HIGHLIGHTS OF RESEARCH PROGRESS
NATIONAL INSTITUTES OF HEALTH
1957

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

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Items of Interest on Program Developments and
Research Studies Conducted and Supported by the
Institutes and Divisions of NIH

As Presented to the
Congress of the United States

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

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INTRODUCTION

This document is a compilation of statements by the Institutes and Divisions of the National Institutes of Health. The statements have been submitted to Congress at hearings on appropriations to the Department of Health, Education, and Welfare for fiscal year 1959. As in past years, the Office of Research Information, NIH, anticipating publication in the records of the Congressional hearings, has prepared this compilation to meet requests and for administrative use.

In no sense is Highlights a comprehensive review of NIH activities. It contains representative items selected in light of both scientific importance and public interest. Undoubtedly many results of equal or greater significance are omitted.

The items for the most part concern the work of NIH staff scientists, laboratory and clinical. Also included are research accomplishments of many NIH grantees, but no attempt has been made to reflect the relative magnitude of the grants program. During the period covered--calendar year 1957--more than two-thirds of NIH funds for research were granted to investigators in universities, medical schools, and other non-Federal research centers.

The items in this report are based on published work. The scientists' articles appear in a wide range of journals, mainly non-Federal, which are available in medical libraries. Further information, including references to these articles, may be obtained through the Office of Research Information, NIH, or the information office of the Institute concerned.

HIGHLIGHTS OF PROGRESS

IN RESEARCH

ON CANCER

1957

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Cancer Institute

Research during the past year has continued to narrow the gap between cancer and man's ability to control it. Advances in knowledge of the nature of the disease and progress in diagnosis and treatment have been accomplished through the variety of scientific disciplines that make up cancer research. This entire research effort is unified by the constant, single goal --the eradication of cancer in man.

Studies of cancer causation are spurred by the hope that identification of causative agents and knowledge of the carcinogenic process may lead to prevention. In 1957, information on this facet of the problem was highlighted by findings obtained in virus studies. In studies of diagnosis, additional information that confirmed the advantages of using the cytologic technique for the detection of cancer of the uterine cervix was reported. Investigations on the treatment of cancer, especially by chemotherapy, remain a bright spot in research. The success in treating a rare tumor by drug therapy appears to reaffirm the belief that cancer may eventually be controlled by the use of drugs.

The items presented in this report were selected as representative of the highlights of progress in research studies conducted and supported by the National Cancer Institute. They include significant advances in many areas of clinical and laboratory investigation. National Cancer Institute staff scientists are identified by the Laboratory or Branch with which they are connected. Grantees are identified by their institution. Only the name of the senior author of the report is usually given.

CAUSATION OF CANCER

Studies on the causation of cancer have several objectives. One is the identification of cancer-producing agents or factors in the environment and the individual, and the study of their effects. Another is an understanding of the process by which carcinogens cause cancer. Still a third objective is the prevention of cancer by elimination of carcinogenic hazards in the environment and by identification of possible natural defense mechanisms against cancer. An implication of this research is that, if the factors controlling natural immunity can be identified, the means of inducing resistance to cancer, possibly through the development of vaccines becomes a possibility.

Important among the agents that cause cancer in animals are viruses. There is little evidence that cancer in man is associated with viruses. However, advances over the past several years in this area of research--discovery of many hitherto unknown human viruses, better knowledge of the properties of viruses, and new findings about the chemistry of nucleic acids, the complex molecules that are important components of both viruses and living cells--have contributed to intensive studies of viruses as cancer-inducing agents. These studies have been aided also by the availability of many kinds of human cells in tissue culture, with the result that, on the assumption that cancer is a disease of individual cells, new test systems for possible cancer viruses are provided.

NEW HOST-ROUS SARCOMA VIRUS RELATIONSHIPS ARE ESTABLISHED

One approach to an understanding of the way in which a cancer virus attacks living cells is a quantitative study of host reactions to a known virus. The most widely investigated virus tumor is the Rous sarcoma, which was described as a transplantable neoplasm of fowl in 1910 by Dr. Peyton Rous. One year later, he described a filterable agent which was separable from the tumor and capable of transmitting it.

Dr. W. Ray Bryan, Laboratory of Biology, has reported results of extensive quantitative studies of the host response in relation to the inducing dose of Rous sarcoma virus. The following "biological manifestations" of the cancerous reaction were measured: incidence of tumors; latent period, or tumor-induction time; rate of growth of tumors; structural characteristics, or "type" of tumor; amount of virus recoverable from the tumor tissue; time-to-death of hosts with tumor; and, suggestively, but not yet conclusively,

tendency toward metastasis, or spread to other sites.

The results obtained in this study have established the following host-virus relationships: 1) Rous sarcoma virus is itself the direct cause of the cancer and is not merely the agent that acts indirectly by "triggering" some inherent characteristic within the host; 2) the biological properties of Rous sarcomas are related to the dose of the Rous sarcoma virus used to induce the tumors; 3) the quantity of virus demonstrable in Rous sarcomas is a function of the dose of virus used to initiate them, and at extremely low initiating dose levels the resulting tumors may show no demonstrable virus on extraction. This is contrary to the common assumption that tumors having a viral origin should yield demonstrable virus on extraction, and that failure to do so represents evidence that they are of "nonviral" origin.

ACTIVITY OF PAROTID TUMOR AGENT INCREASED IN TISSUE CULTURE

Studies of other possible cancer-inducing viruses are being carried out by Dr. Sarah E. Stewart, Laboratory of Biology, who reported earlier that injection of new-born mice with cell-free mouse leukemia extracts produced, not leukemia, but an unusual type of tumor of the parotid (salivary) gland that occurs spontaneously in mice only rarely; and that injection of new-born mice with cell-free extracts prepared from the parotid gland tumors did not produce neoplasms. Dr. Stewart now reports on results of a collaborative project with Dr. Bernice E. Eddy, Division of Biologics Standards, in which cell suspensions of parotid gland tumor tissue were maintained serially in tissue culture. When supernatant fluids from the cultures were injected into new-born hybrid mice (produced by crossing two inbred strains), the mice developed tumors. All mice that developed such tumors had primary parotid gland tumors and some also developed tumors of the thymus, adrenal glands, and mammary glands. Similar results were obtained from cultures of a spontaneous leukemia.

In an extension of the work, Dr. Stewart found that non-inbred (Swiss) mice are also susceptible to the tumor agent. A cell-free mouse leukemia extract was inoculated into tissue culture and incubated for 2 weeks. Inoculation of supernatant fluids from the cultures into Swiss mice less than 24 hours old produced parotid gland and other tumors.

By use of the tissue culture technique the activity of the tumor agent has been so increased that in some instances inoculated mice develop tumors in 6 weeks.

CELL-FREE MOUSE LEUKEMIA EXTRACTS PRODUCE LEUKEMIA, OTHER CANCERS

Some investigators have found that inoculation of newborn mice with cell-free mouse leukemia extracts results in the appearance some months later of leukemia, as well as parotid gland tumors. Dr. Anna Dean Dulaney, University of Tennessee, has reported the following result: Injection of cell-free leukemic extracts from a high leukemic inbred strain (AK) of mice into newborn mice of low leukemic strains (C3H) results in a significant increase in leukemia, parotid gland tumors, and sarcomas as these animals age, and that the leukemias which develop are C3H, rather than AK, in character. The active portion of the extract which produces leukemia and other neoplasms has not been characterized. It may be a virus, an accelerating factor, or an agent with some other property. Dr. Dulaney concludes, however, that the agent that incites parotid gland tumors is different from that which causes leukemia.

MOUSE SEX GLAND TUMORS CONTINUE TO GROW WHEN STIMULUS IS REMOVED

Another possible cause of cancer under investigation is abnormal hormone production. In one study, scientists of the Laboratory of Biology undertook to determine the extent to which the development and transplantation of tumors of the testes induced in mice by administration of a female sex hormone were dependent on continuous hormone stimulation. Dr. Howard B. Andervont, Chief of the Laboratory, has reported that such tumors--which until now have been considered dependent for further growth upon continued hormonal stimulation--appear to have only limited dependence and can progress to independence when the stimulus is removed or when the tumor is transplanted into new hosts.

When testicular tumors were induced by insertion of pellets containing stilbestrol under the skin of mice, some tumors continued to grow after the pellets were removed and some tumors regressed. When a second pellet was given to mice whose testes had receded to normal, the tumors recurred promptly. In other experiments in which the first stilbestrol pellet was removed before development of testicular tumors, administration of a second pellet resulted in tumors in a short time. These results showed that changes initiated by hormonal stimulation persist for a long time, and suggest that the changes are irreversible but not sufficient for further progression unless the mice received a second hormonal stimulus. Transplantation of testicular tumors that arose in the presence or absence of stilbestrol pellets or those that continued to grow upon removal of pellets also resulted

in the production of many hormone-independent tumors.

NON-HORMONAL CHEMICALS CAUSE HIGH INCIDENCE OF RAT PITUITARY TUMORS

The prolonged, continuous treatment of rats and mice with female sex hormones has long been known to induce tumors of the pituitary, the master endocrine gland located at the base of the brain. Now, Dr. Harold P. Morris, Laboratory of Biochemistry, has reported the discovery that chemicals simpler in structure than hormones can cause a high incidence of pituitary tumors in rats.

Three aromatic amines with sex hormonal activity -- para-fluoroacetanilide, orthohydroxyacetanilide, and 3,4-dimethylaniline -- produced these results when added in small amounts to the diet of rats for 9 to 15 months. After this time, the chemicals were withdrawn, and the animals were kept under observation until they were sacrificed before they were 21 months old. The animals receiving the chemicals developed 73 percent to 85 percent incidence of pituitary tumors, three times as many such tumors as the animals fed the same food without the chemicals. Spontaneous tumors of the pituitary are common in very old rats. Most of them, however, develop in animals over 20 months old.

The finding that the three chemicals studied produce primarily only tumors of the pituitary, the endocrine gland that controls many endocrine secretions, provides investigators with a new approach to studies of cancer causation in laboratory animals.

One of the carcinogenic chemicals that causes in rats a variety of tumors, such as tumors of the liver, bladder, breast, and auditory canal, is N-2-fluorenylacetamide. Information about metabolites (breakdown products) following administration of this drug in the diet provides a clue to the chemical changes that the carcinogen undergoes in the tissues of the host and hence to the actual form in which the carcinogen may exert its effect.

EFFECTS OF CHEMICALS STRUCTURALLY RELATED TO CARCINOGEN STUDIED

In a project concerned with the chemical changes that N-2-fluorenylacetamide undergoes when ingested by rats, the carcinogenic effect of some chemicals closely related

in structure to the carcinogen have been studied by scientists in the Laboratory of Biochemistry. Dr. Morris has reported that animals fed two of the compounds, N-2- and N-4-biphenylacetamide, developed cancers of the breast and uterus, but no liver tumors. All 16 animals fed a third compound, N-4-(4'-fluoro)biphenylacetamide developed a variety of tumors. The sites included the breast, kidney, ear, uterus, small bowel, liver, and spleen. This is the first report of a high incidence of kidney tumors in rats after ingestion of this compound. Almost half the animals developed kidney tumors; often they were multiple and in 1 animal they were bilateral.

RAT, GUINEA PIG MAY METABOLIZE CARCINOGEN DIFFERENTLY

Dr. John H. Weisburger, Laboratory of Biochemistry, has reported results of still another project on the metabolism of N-2-fluorenylacetamide. The study was undertaken to determine, if possible, why a chemical known to cause cancer in rats does not cause the disease in guinea pigs. The carcinogen was specially prepared so that it contained a radioactive carbon atom and was shaped into pills, which were fed to guinea pigs. After a suitable interval, tissues, blood, and excreta were tested for radioactivity. The materials in which radioactivity were detected were anlyzed and the individual metabolic products identified.

About 12 metabolic products of the carcinogen ingested by rats have been identified; any one or several of these may be the actual material that causes cancer in rats. Not all these substances are produced by guinea pigs, and it is concluded that the metabolism of the carcinogen in the guinea pig is different from that in the rat. The implication of these results is that the compound or compounds present in the metabolic products of the rat, but lacking in those of the guinea pig, may be responsible for the carcinogenic action of N-2-fluorenylacetamide.

Laboratory investigation of substances suspected of being carcinogenic provides opportunities to study the chemistry of these materials and their effect in animals. Some of those studied were found in the environment of work or daily living, and others were obtained from cancer patients.

CHROMITE, CHROMIUM DUSTS BECOME SOLUBLE IN LABORATORY TESTS

In laboratory investigations of the possible causes of the excessively high lung cancer

incidence among workers in the chromate-producing industry, scientists of the Environmental Cancer Section have observed that dusts of crude chromite ore and metallic chromium suspended in various liquids become soluble when aerated. Dr. Charles H. Grogan has reported this finding, which leads the investigators to suspect that body chemicals dissolve chromite ore and metallic chromium when they are inhaled as dusts. The dusts may form a reservoir from which dissolved chromium may be released in small amounts over long periods. The cells surrounding the reservoir would be bathed in abnormally high concentrations of chromium. Additional experiments in animals in which chromite-ore dust was introduced into the lungs demonstrated that chromium was carried into other organs. The amounts of chromium found were of the same order of magnitude as those recorded in the literature for analyses of tissues, blood, and urine of exposed workers. It is concluded that exposures to fine dusts of chromite ore and metallic chromium represent a potential hazard of "chronic nature."

MOUSE EXPERIMENT SHOWS DELAYED CANCER ACTION IN CIGARETTE TAR EXPOSURE

Dr. E. V. Cowdry, Washington University, reporting on studies begun by the late Dr. Evarts A. Graham, has given additional information on the carcinogenic effect of cigarette tar in mice. Seventy-four mice were painted with cigarette tar for 12 months; then the painting was stopped in half the mice and continued in the other half. It was found that a significant number of mice in both groups developed cancers of the skin beginning at about the fourteenth month. The mice developed cancers more frequently and more quickly in the group in which painting was continued than in the other group. These findings show the existence of a lag period after exposure to cigarette tar has been stopped. The results seem to confirm the clinical observation that a cigarette-induced bronchial cancer can appear even though the patient stopped smoking a few years earlier.

EXCESSIVE PROLIFERATION OF CONNECTIVE TISSUE IS PRECANCEROUS IN MICE

In experiments on the induction of cancers by plastic films embedded under the skin of rodents, Dr. B. S. Oppenheimer, College of Physicians and Surgeons of Columbia University, observed that the film always became encased in a pocket of fibrous tissue and that cancers developed within it one or two years later. To learn what precancerous process, if any, preceded the appearance of the cancers, 200 polystyrene embeddings in rats were removed from their pockets at monthly

intervals. When they were removed less than 6 months after embedding, no tumors were produced. If the plastic films remained longer, the usual number of tumors appeared; but if the pockets of fibrous tissue were removed also, no tumors developed.

The investigator's attention was therefore directed to the pocket as an essential factor. A series of 120 pockets was removed at intervals of 1 to 30 months from rats embedded with polystyrene or other films. Microscopic study showed formation of varying amounts of connective tissue cells in the first six months. Subsequently, more of these cells, and sometimes microscopic nodules, were seen. As the time approached 1 year and thereafter, there appeared still larger nodules or thickenings, in which some of the cells showed precancerous changes or small areas of cancer. These findings persuaded Dr. Oppenheimer that the precancerous stage is an excessive proliferation of fiber cells which eventually become cancerous.

HAMSTERS GIVEN CANCER PATIENTS' BILE DEVELOP PRIMARY CANCERS

The possibility that cancer of the biliary tract is caused by a cancer-causing compound in bile is apparently supported by results of experiments on hamsters performed by Dr. Joseph G. Fortner, Sloan-Kettering Institute for Cancer Research. Primary cancers (adenocarcinomas) of the small intestine with spread to lymph glands have been found in three hamsters that were injected under the skin with bile obtained from living patients with cancer of that part of the biliary tract (bile ducts) which lies outside the liver. A fourth animal had a cancer of the colon. Dr. Fortner concludes that the observed cancerous activity may be the manifestation of some abnormal compound formed or excreted by the liver.

INCIDENCE OF UTERINE CANCER IS COMPARED

Another aspect of the investigation of environment involves epidemiological studies. These studies provide data on prevalence, incidence, distribution, and mortality of cancer, and contribute to the knowledge of the nature and extent of cancer in the population.

A collaborative study by Dr. Lucia J. Dunham, Laboratory of Pathology, and Dr. Harold F. Dorn, Chief, Biometrics Branch of the National Institutes of Health, was made to compare the incidence of cancer of the uterus (womb) among different population groups. They found that in white women living

in New York City, cancer of the uterine cervix (neck of the womb) occurs 3 to 4 times as frequently in non-Jewish as in Jewish women. Jewish women in New York City and in Israel have the same incidence of the disease. The incidence of uterine cervical cancer among Jewish women was 4.8 per 100,000. Among non-Jewish women in New York City, it was 17.3; and in selected urban areas in the United States, it was 39.1. In population groups other than Jewish studied in New York City, Puerto Rican women had the highest incidence of uterine cervical cancer--111.3 per 100,000. The rate for non-white women was 54.5.

CHILDHOOD LEUKEMIA MAY BE RELATED TO EXPOSURE OF MOTHER TO CARCINOGENS

In another epidemiological study, 323 mothers of patients at the Children's Cancer Research Foundation, Boston, were interviewed regarding their medical, occupational, and personal histories. The women were mothers of leukemic children and mothers of children with other cancers. Fifty mothers whose children are being treated for other diseases at the Orthopedic Clinic, Boston, were interviewed as controls. Early findings are reported by Dr. Miriam D. Manning, Field Investigations and Demonstrations Branch.

Some 32.4 percent of the mothers of leukemic children reported histories of allergy, including hives and hay fever, compared with 11.8 percent of mothers of children with other cancers, and 20.0 percent of the control mothers. Some 19.5 percent of the mothers of leukemic children had received X-ray therapy before or during pregnancy with the leukemic child, compared with 12.2 percent of mothers of children with other cancers, and 11.9 percent of the control mothers. Both groups of mothers of cancer patients were higher than controls in industrial and home exposure to possible carcinogens, such as benzol, insect sprays, floor and furniture wax.

The most significant findings of this study are an increased incidence of allergy and more frequent exposure to therapeutic X-rays among mothers of leukemic children. This investigation will continue as more patients become available, and more information on the association between leukemia in children and environmental factors will be obtained.

CANCER AMONG MINORITY GROUPS IN U.S. STUDIED

Studies were continued by the Field Investigations and Demonstrations Branch of cancer among minority groups in the United States. Dr. Robert L. Smith has now reported on an analysis of recorded mortality among a group of Hawaiians and Filipinos of Hawaii and a

mixed racial group in the United States composed largely of Filipinos. The values obtained were compared with expected death rates among the white or non-white populations of the United States. The main difference between the two groups was a marked excess of recorded mortality for cancer of the liver among the male Filipinos of Hawaii, as compared with the deaths expected; a smaller excess among the group residing in the United States was not statistically significant.

Cancer research scientists believe that identification of substances or factors in the environment that may increase the risk to human cancers can permit the development of practical means of eliminating such hazards and thereby increase the possibilities for preventing certain types of cancer.

ROUTINE REMOVAL OF SKIN MOLES CONTRAINDICATED

A procedure that has been advocated in the last several years for prevention of melanoma is routine removal of skin moles. Dr. Eugene J. Van Scott, General Medicine Branch, now reports that routine removal of such moles is contraindicated. This conclusion is based on the evidence obtained in studies of the moles occurring on the palms (palmar) and soles (plantar) of a large number of individuals.

These moles were selected for study because of the general belief that chronic irritation of moles predisposes them to develop into melanoma and that the moles of the soles are disproportionately susceptible to malignant change. A total of 735 individuals ranging in age from 6 months to 81 years was studied. Among them were normal individuals and hospitalized patients with a variety of illnesses, including several types of cancer. Of all the subjects examined, 195, or 26.5 percent, had at least one palmar or plantar pigmented lesion. These results show that palmar and plantar moles are not as uncommon as has been generally believed. Forty-five pigmented lesions removed from 40 adults were clinically indistinguishable. They had no obvious characteristics on the basis of which their histological types could be predicted.

Dr. Van Scott acknowledges that moles are undeniably implicated in the development of melanoma. He points out, however, that according to other investigators' results, 16 to 30 or more moles occur on the entire skin of an adult and that it cannot be predetermined which individual mole will undergo the malignant change. Hence, the prophylactic removal of these lesions is an impractical, unrealistic

measure for preventing the proportionately few melanomas that could be expected to arise from this large number of nevi.

OROTIC ACID REDUCES CANCER-PRODUCING EFFECT OF METHYLCHOLANTHRENE

A possible way of preventing cancer is suggested by the work of Dr. Stanfield Rogers, Duke University School of Medicine, who has reported that the cancer-producing effect of the chemical, methylcholanthrene, is reduced in mice by orotic acid, a naturally occurring chemical. In studies of the mechanism by which methylcholanthrene produces cancer in mice, Dr. Rogers injected a group of mice intramuscularly with methylcholanthrene, then supplemented the diet of half the mice with orotic acid. The rest of the mice were the controls and received tap water instead of orotic acid. He found that the mice receiving supplementary orotic acid developed fewer lung tumors than did the controls. He also found that in both groups the number of surviving animals and the average size of the tumors were essentially the same. These findings indicate that orotic acid exerts its inhibitory influence at the level of initiation of the tumors rather than upon their subsequent growth.

Orotic acid is known to play a large part in nucleic acid synthesis. Hence, Dr. Rogers suggests that methylcholanthrene initiates the neoplastic change by processes closely related to the synthesis of nucleic acid. Since the biological characteristics of cancer cells are thought to be determined by their nucleic acid composition, the results of this study appear to emphasize the importance of continued research on the processes by which nucleic acids are synthesized.

Another facet of the problem of the causation of cancer concerns the possible existence of natural defense mechanisms--immunity--against cancer. Information on this aspect of cancer research has been produced by the studies summarized in the following paragraphs.

AGE AN IMPORTANT FACTOR IN IMMUNOLOGICAL STUDIES

In a study of leukemia in guinea pigs, Dr. Eli M. Nadel, Laboratory of Pathology, reports that he successfully transplanted acute leukemia from a highly inbred strain of guinea pigs to 7-day-old non-inbred guinea pigs. The leukemia was then carried in transplant in very young non-inbred guinea pigs for over

18 generations and grew progressively when transplanted back to the original inbred strain. "Takes" of transplants were more frequent in animals under 10 days of age (89 percent) than in animals between 3 and 7 weeks of age (31 percent) and no "takes" occurred in animals over 8 weeks of age.

These results show that age appears to be an important factor in the operation of the immunogenetic barrier: that is, the resistance to transplants between genetically unrelated members of the same species. This evidence is particularly significant in that it focuses on age as a factor to be studied more intensively in attempts to understand how the immune response develops.

INFECTION AND TUMOR DAMAGE FACTORS APPEAR RELATED

A possibility that resistance or susceptibility to infection and to cancer involve similar factors has been suggested by the observation that some factors involved in infection and in tumor damage appear to be related. Dr. Maurice Landy, Laboratory of Chemical Pharmacology, has reported results of a comprehensive, systematic investigation of the biological properties of complex polysaccharides from various sources. Polysaccharides are complex sugars of high molecular size, isolated many years ago from gram-negative bacteria. They not only produce reactions characteristic of infectious bacteria, but also damage cancer tissue.

The results of the study show that regardless of the source of the polysaccharides--bacteria, plant tissues, or normal or cancerous animal tissues--they all produced in laboratory animals the reactions hitherto considered characteristic of bacterial polysaccharides. Twelve reactions were studied, including fever, altered white blood cell count, Shwartzman phenomena, changes in blood properdin (a substance normally present in blood serum which may aid the body in resisting infections by some bacteria and viruses) levels, altered resistance to infection, tumor damage, and shock. These findings suggest that these polysaccharides constitute an underlying feature common to many situations injurious to man, such as infection and cancer.

The possible existence of natural immunity against cancer raises the question of whether antisera effective in inducing resistance may be developed. When tumor tissue from an animal is injected into an animal of another species, the blood serum of the latter forms antagonistic substances

to fight and destroy the introduced tumor. The tumor material is known as the antigen, and the blood-formed substances are antibodies. These reactions would form the basis for the development of vaccines.

HUMAN CANCER INJECTIONS IN RATS PRODUCE ANTIBODIES AGAINST TRANSPLANTS

Results of a study by Dr. Helene W. Toolan, Sloan-Kettering Institute for Cancer Research, showed that substances in the blood and tissues of laboratory animals injected with human cancer tissue destroy the cancer cells and subsequently prevent successful transplantation of the human cancer tissue. In the study, laboratory rats were injected with minced human cancer tissue. Seven days later, blood and tissue samples were taken from these animals. Fresh human cancer tissue was exposed to these blood and tissue samples for one hour and then implanted into rats pretreated with cortisone and/or x-ray. Dr. Toolan had previously found that human cancer will grow in laboratory animals pretreated in this manner. In the present experiment, the human cancer tissue failed to grow. Cancer exposed to blood and tissue of uninjected rats grew readily in the pretreated animals.

The cell-destroying agents were found in the blood, spleen, lymph nodes, and thymus of the injected animals but were not found in muscle, heart, kidney, or lung. This finding confirmed the concept that the body's natural defenses are associated with lymphoid cells (white cell elements).

VACCINE DEVELOPED AGAINST LEUKEMIA-LIKE DISEASE IN MICE

Dr. Charlotte Friend, Sloan-Kettering Institute for Cancer Research, has reported the development of a vaccine against a disease with the characteristics of leukemia, transmissible to adult mice by a cell-free agent, which she discovered a year ago.

When mice were injected with the virus, over 80 percent developed the disease in two or three weeks. The rest showed immunity to infection by the virus. To determine whether this resistance was innate or had been built up as a result of the first injection, a study of the antigenic properties of the agent was undertaken and the vaccine was developed. It is a formalin-killed virus preparation. When mice were given a series of three injections of vaccine at weekly intervals, and then given live virus, about 80 percent of the animals proved to be immune even as long as four weeks after vaccination.

CHARACTERISTICS OF CANCER

Knowledge of the characteristics of cancer is important in providing practical information about the behavior of a cancer and its effect on the patient, and fundamental information about the nature and development of cancers. Intensive efforts to discover important characteristics among cancers grown under controlled conditions have led to many advances in knowledge. They have also brought to light the need for additional information about the nature and characteristics of normal growth processes. As a consequence, cancerous and normal growth processes are being studied simultaneously by application of the techniques of the many biological, chemical, and physical sciences that constitute cancer research.

Tissue culture is a valuable tool in cancer research because it permits the study of cells grown outside the body. Thus, tissue culture facilitates the study of the development of malignancy, effectiveness of anticancer drugs, and differences between normal and cancer cells. The usefulness of this tool has been increased by technological advances, which have made possible the growth and maintenance for long periods of relatively large quantities of cultures under controlled conditions and the establishment of pure strains of cells (clones) each grown from a single cell.

EGG EXTRACT FOUND CHEAP, SATISFACTORY SUBSTITUTE IN LONG-TERM CULTURES

Scientists of the Laboratory of Biology are engaged in studies of nutrient media for tissue culture, in order to obtain reproducible and adequate media. Development of such media would give the investigator control of the nutrition of cells, and so possibly permit him to influence growth, metabolism, function, and specific differentiation.

Dr. Virginia J. Evans has reported that unincubated whole egg extract ultrafiltrate from either fertile or nonfertile eggs is a satisfactory substitute for the more expensive 10-day chick embryo extract in promoting growth and long-term cultivation of several cell types in tissue culture. Large volumes of extract required for cultivation of massive cultures maintained in a rapidly agitated suspension have thus been made available and are relatively inexpensive for this use.

SEVERAL PURE CLONES OF MOUSE CELLS ADAPTED TO PROTEIN-FREE MEDIUM

In the Laboratory of Biology, a pure strain of mouse fibroblast cells from NCTC clone 929 (strain L) has been established and maintained for almost three years in an entirely chemically defined, protein-free medium, designated as medium NCTC 109. Proliferation of the cells in this medium during the last year has been sufficiently rapid to allow weekly serial subculture. However, the original adaptation of cells of clone 929 from a nutrient medium rich in proteins to the chemically defined, protein-free medium required careful nurturing for six months, before there was assurance that the cultures would continue to proliferate rapidly and could be readily subcultured, and thus considered stabilized. William T. McQuilkin has now reported the successful adaptation of several additional cultures of clone 929 cells to medium NCTC 109. The availability of cell strains well adapted to and stabilized by maintenance in a chemically defined medium makes possible controlled and reproducible studies of the behavior and characteristics of cells, cell changes, the carcinogenic process, and susceptibility of cells to therapeutic agents.

Cultures of pure strains have been studied in the Laboratory of Biology for the past 11 years. They have provided a basis for accurate comparison of experimental data obtained in laboratories in different parts of the world. In the last few years, for example, "starting cultures" of one pure strain of mouse cells have been shipped to such research centers as the Pasteur Institute in France, University of Glasgow in Scotland, University of Toronto in Canada, University of Tokyo in Japan, and University of Stockholm in Sweden.

MOUSE LIVER CELL CLONE MAINTAINED FIVE YEARS

Establishment of a clone of mouse liver cells has been reported by Mrs. Gwendolyn L. Hobbs. This clone was established from a single cell isolated from a strain adapted to grow in chicken plasma clotting mixture and has been maintained in culture for five years. Cells of both the parent strain and the derived clone show no appreciable differences in gross morphology or in general culture architecture.

TWO CLONED STRAINS OF HUMAN EPITHELIAL CELLS ESTABLISHED

A project to grow cells from human skin is being carried out in collaboration with the Tissue Bank of the National Naval Medical Center. A result of this project reported earlier has

been the isolation of a strain of human epithelial cells. This strain has been maintained in culture for almost four years.

If successful therapy is to be given to a patient who is critically burned or whose skin is otherwise denuded, a better understanding of the cells of such strains is necessary. Vernon P. Perry, of the Tissue Bank, and Dr. Katherine K. Sanford, of the National Cancer Institute's Laboratory of Biology, have reported the successful cultivation of two cloned strains of these human epithelial cells and have described the in vitro behavior of these clones.

SEVERAL NEW HUMAN CELL STRAINS REPORTED

Another collaborative study, reported by Dr. Samuel Baron, Division of Biologics Standards, and Dr. Alan S. Rabson, National Cancer Institute's Laboratory of Pathology, has produced a tissue culture strain of human epithelial-like cells derived from a lung cancer. The isolation, maintenance, and morphological features of this cell strain have been described. Preliminary viral studies indicate that these cells are susceptible to several viruses, including poliovirus and Coxsackie B2 virus.

Dr. Jerome T. Syverton, University of Minnesota, has reported the establishment of three new human strains of palate cells, esophageal lining cells, and liver cells. Patient biopsy material provided the source tissues. The cultures were grown in a medium consisting of 20 parts of compatible human serum, 76 parts of a yeast extract basal medium, and 4 parts of sodium bicarbonate solution. Dr. Syverton reported also that a clonal or variant strain subline of esophageal cells has been derived.

NORMAL, CANCER CELL LINES DIFFER IN RESPONSE TO HYDROCORTISONE

A tissue culture study has enabled scientists of the Laboratory of Chemical Pharmacology to recognize differences between normal and cancer cell lines. It has previously been reported in the literature that human cell strains grown in tissue culture from normal and cancer tissues are similar in appearance and biologic behavior. Dr. Ira Kline has reported that, in a study of the response of four human cell strains to hydrocortisone, normal cells were more resistant to the drug than cancer cells. The findings in this study are of particular interest to tissue culture studies of the anticancer action of adrenal cortical hormones, which are produced by the adrenal glands.

NORMAL, CANCER LINES DIFFER IN GROWTH ABILITY WHEN TRANSPLANTED

A study reported by Dr. G. E. Foley, Children's Cancer Research Foundation, showed another difference between normal and cancer cell lines grown in tissue culture. In this investigation, the cell lines were similar in biochemical and morphological properties and in susceptibility to certain anticancer agents. However, they differed significantly in their ability to grow when transplanted into the cheek pouch of normal hamsters. Tests with 14 tissue culture cell lines produced the following results: 1) all cell lines grew when one million cells were implanted; 2) the cell lines derived from cancerous tissue produced tumors when only 10,000 cells were implanted; 3) when the hamsters were conditioned with cortisone acetate, all cell lines grew when 100,000 cells were implanted, and cancer cell lines grew when only 1,000 cells were implanted.

TRANSPLANTABLE RAT TUMORS METASTASIZE

Transplantation of tumor tissue is another technique by which living cancer tissue is made available for study outside the body. Dr. A. S. Mulay, Laboratory of Pathology, has reported the occurrence of transplantable rat tumors that metastasize. The tumors were produced in rats by weekly subcutaneous injection of the chemical, para-dimethylaminobenzene-1-azo-1-naphthalene, which is similar to a carcinogenic compound commonly used to develop liver tumors in rats. Most of the tumors arose at the site of injection and were frequently multiple. Some of them were successfully carried through several transplant generations and, after the second passage, usually metastasized to the lungs.

HUMAN TUMORS THRIVE IN LABORATORY ANIMALS

Dr. Helene W. Toolan, Sloan-Kettering Institute for Cancer Research, has reported the establishment of three human tumors that can be transplanted and maintained in suitably conditioned hamsters and rats. The tumors include a cartilaginous type of tumor, a tumor obtained from the outermost tissue of the tongue, and a cancer derived from the human colon. All three are vigorous, rapidly growing, and uniform tumors. As an example, the cartilaginous tumor is now in its 30th transplant generation. It increases twentyfold in about 40 to 50 days in both hamsters and rats, and in either animal it is a reliable and uniform tumor for testing of chemotherapeutic agents.

Knowledge of the effects of radiation on living tissue is particularly important in cancer research. Such information contributes to improved therapy and diagnosis as a result of advances in techniques of producing and administering radiation. At the same time, such information can make possible the development of techniques for protection against injury from radiation exposure.

MICE IRRADIATED AT 710 R UNPROTECTED BY RAT BONE MARROW

Several years ago National Cancer Institute investigators reported results of studies showing that mice exposed to about 900 roentgens (supralethal dose) of total-body radiation recovered from the effects of radiation injury when given an intravenous injection of rat bone marrow after irradiation. Some mice died more than 30 days later. This was due, it was suspected, to recovery of the host animal's bone marrow from the radiation damage, resulting in a host-antibody reaction to the transplanted heterologous bone marrow (marrow from a different species).

A group of investigators, including Dr. C. C. Congdon, Oak Ridge National Laboratory, and Dr. N. Gengozian, a Research Fellow of the National Cancer Institute, reasoned that if these suspicions were correct, a lower dose of radiation (710 r) would produce less injury to the host mouse's bone marrow and still permit successful transplantation of the rat bone marrow. Thus, quicker recovery and more violent immune response would occur.

In the study now reported by Dr. Congdon, all mice exposed to 710 r of total-body radiation and injected with rat bone marrow died in 16 days. Their bone marrow showed an early recovery of all blood-forming elements and then abrupt, total destruction of the marrow 6 to 9 days after irradiation. Mice exposed to 710 r and injected with isologous bone marrow (marrow from the same strain of mice) did not die. Thirty percent of the mice exposed to 710 r but receiving no marrow injection died in 30 days. Thus, the anticipated result was confirmed by the experiments.

OWN MARROW PROTECTS FIRST GENERATION MICE PROGENY BETTER THAN PARENTS'

Investigators of the Laboratory of Biology are attempting to learn what genetic factors are involved in influencing the ability of injected normal bone marrow to protect the irradiated subject. The first of a series of papers on the findings

of this project has been published. Miss Delta E. Uphoff has reported that when first generation progeny (F_1) of two inbred strains of mice were exposed to a lethal dose of X-rays, the inoculation of F_1 marrow afforded the irradiated mice better protection than bone marrow from either of the parent strains. Bone marrow of the parental strains gave the progeny only transient protection, and the mice died from the effects of a late irradiation reaction.

RADIATION DAMAGE TO SCALP HAIR ROOTS CORRELATED WITH DOSE

Dr. Eugene J. Van Scott, General Medicine Branch, has reported on a study of the effect of radiation on hair roots of the human scalp. A group of patients under study for neoplastic diseases received either X-irradiation of scalp areas or electron irradiation of the entire body surface, including the scalp. Hairs were pulled from the scalp, and the hair roots were examined microscopically. The results show that radiation has a direct effect on scalp hair roots and that the extent of damage is correlated with the radiation dose.

Evidence of a direct effect of radiation was found in the following observations: 1) the roots of growing hairs showed abnormal changes as early as 4 days following irradiation; 2) progressive atrophy of the hair bulb at the base of the root occurred; 3) the number of growing hair roots affected was proportionate to the dose of radiation sustained by the hair roots and to the interval between irradiation and examination.

These observations suggest the possibility that radiation exposure can be estimated quantitatively in man. Reprints of the paper have been requested by the United Nations Scientific Committee on the Effects of Atomic Radiation.

RADIOACTIVE SULFUR SLOWS CARTILAGE GROWTH, HAS THERAPEUTIC POSSIBILITIES

Scientists of the Radiation Branch investigated the effects of radioactive sulfur (S^{35}) on growing cartilage, because of the possibility that this substance might be useful in treating patients with chondrosarcoma (cartilaginous tumor). The results of the experiments reported by Dr. Philip Rubin showed that radioactive sulfur arrested cartilage growth in the weaned rat and dwarfed the skeleton of the animal. A second result with possible implications for patient therapy was that a fractionated dosage schedule was more effective in arresting and altering cartilage growth and less toxic than a single injection at the same level.

Dr. Raymond G. Gottschalk and his associates, The George Washington University, have obtained similar results in studies with suckling rats. In a continuation of the studies, Dr. Gottschalk has reported that tracer amounts of radiosulfate are taken up by human cartilaginous tumor and suggests that sufficiently large amounts of the isotope might inhibit the growth of chondrosarcoma. Early results from animal studies indicate that S^{35} moderately inhibits the growth of two transplantable mouse tumors.

RADIATION SENSITIVITY OF LEUKEMIC BLOOD CELLS STUDIED BY NEW TECHNIQUE

Dr. Robert Schrek, Northwestern University, Evanston, Ill., has reported a study on the feasibility and utility of testing the radiation sensitivity of blood cells of patients with the blood cancer (leukemia) using time-lapse cinemicrography and phase microscopy. These methods have been used to measure the effect of X-rays on rabbit and human white cells and other cells. It was found that certain white cells, lymphocytes, died when treated with small doses of X-rays (50 to 1000 roentgens) and showed profound changes in the internal structure of the cell before death. Another variety of white cells, called a granulocyte, obtained from human beings and rabbits, was resistant to large doses of X-rays. Studies were made also on the sensitivity to X-rays of the blood cells from 12 chronic lymphocytic leukemia patients. The blood cells were grown in tissue culture. Non-irradiated leukemic lymphocytes survived 4 to 7 days under the conditions of culture, but dosages of 5 to 1000 r reduced the survival time of blood cells of 11 out of 12 patients. The blood cells of the twelfth patient were resistant to dosages of 1000 r but were killed by irradiation with 4000 r.

CANCER PATIENTS BURN CALORIES FASTER WHEN GIVEN EXTRA FOOD

Nutrition is an important problem in studies of host-tumor relationships. In one study, the effect of feeding a high caloric diet to cancer patients was reported by Dr. Donald M. Watkin, General Medicine Branch. Four patients with different types of cancer and four with chronic illnesses other than cancer were given extra food intravenously in the form of a cottonseed oil emulsion, while maintained on a constant oral diet. The patients who did not have cancer responded to the supplementary food intake with positive caloric balances, and they gained sizable amounts of weight. In contrast, the cancer patients had negative caloric balances and merely increased their rate of calorie expenditure. Despite the added calories, they continued to burn more energy than they received.

During this process, it appears that the cancer patients burned fat and retained water, nitrogen, and phosphorus in such a manner as to change the composition of the total body. When the extra food was withdrawn, the patients without cancer tended to maintain the weight gained, but the cancer patients lost weight rapidly.

TUMOR-BEARING RATS GAIN WEIGHT ON DIET CONTAINING TUMOR TISSUE

Progressive growth of a tumor known as Walker carcinosarcoma 256 causes rats to lose their appetites and to grow tumor at the expense of the body's own tissues. The weight loss from muscle and other normal constituents of the body is reduced but not prevented through maintenance of an adequate dietary intake by tube feeding.

On the assumption that the tumor requires some essential nutrient which can be provided from dietary sources or from normal body stores, scientists of the Laboratory of Physiology undertook to compound and test semi-synthetic diets containing different sources of nitrogen (element needed to build protein). Dr. Florence K. Millar has reported that tumor-bearing rats accepted a semi-synthetic diet using casein as the major source of nitrogen for a longer time than they accepted most other rations, yet the cancerous hosts lost weight. Then the tumor-bearing rats were given another diet similar in caloric value, and nitrogen, carbohydrate, and fat content, but using dried Walker 256 tumor tissue instead of casein as the major source of nitrogen. The rats readily ate this diet and did not lose weight, even though they did not consume more of this diet than the casein diet. The tumors grew at approximately the same rate in rats ingesting both diets, but the initial rate was more prolonged when the ration contained dried tumor. Rats that had lost their appetites on semi-synthetic diets began to eat again when offered the diet containing tumor, and they gained weight.

Work is in progress to identify the materials in the tumor diet responsible for conservation of normal tissues. The results may lead to a better understanding of the nature of cancer and provide a practical means of combating weight loss and wasting of the cancer patient.

TETRACYCLINE FLUORESCES YELLOW IN SOME CANCERS UNDER ULTRAVIOLET LIGHT

Scientists of the General Medicine Branch found that tetracycline, an antibiotic, accumulates in certain cancers and can be detected by a simple laboratory procedure. The

drug localizes in tumor tissue and shows a yellow fluorescence when observed under ultraviolet light. This finding may provide a basis for the development of new techniques in the diagnosis and chemotherapy of cancer.

Observation of a cancer patient led to this study. During the course of his treatment, the patient had received many drugs, some of which were known to show fluorescence under ultraviolet light. Some of his cancer tissue was therefore observed under ultraviolet light to learn whether any of the drugs had accumulated in the tissue and whether the tissue had retained fluorescence. When the yellow fluorescence appeared, those drugs that had been given to the patient were tested in tumor-bearing mice. This screening revealed that tetracycline and its related compounds, aureomycin and terramycin, caused fluorescence.

Studies of this phenomenon reported by Dr. David P. Rall showed that in mice autopsied 24 hours after tetracycline was given by injection or mouth, the yellow fluorescence appeared in both normal and cancer tissue. However, in mice autopsied 2 days to 3 weeks after administration of the drug, the fluorescence persisted in the cancer tissue. The only normal tissues in which fluorescence remained were the teeth and growing bones.

FLUORESCENCE DUE TO UNCHANGED DRUG

Results of biochemical studies to determine what happens to the antibiotic, tetracycline, when it accumulates in tumor tissue have been reported by Dr. Ti Li Loo, General Medicine Branch. The evidence shows that the yellow fluorescence seen in mouse sarcoma tissue under ultraviolet light is attributable to unchanged tetracycline. However, the drug probably does not exist as such, but rather in a weak chemical combination with a peptide normally present as a constituent of the experimental tumor tissue.

STUDY MOUSE PLASMA CELL NEOPLASMS RESEMBLING HUMAN MULTIPLE MYELOMA

In another approach to knowledge of the characteristics of cancer, scientists of the Laboratory of Biology and General Medicine Branch are collaborating in studies of mouse plasma cell neoplasms. These cancers are particularly interesting because of their resemblance to human multiple myeloma, an unusual cancer of the bone marrow cells. Dr. Michael Potter, Laboratory of Biology, has reported data on the characteristics of one of these neoplasms which was successfully transplanted and found to be similar to multiple myeloma of man in microscopic appearance of tissues, in development of bone lesions, and in

production of an abnormal blood serum protein.

MOUSE SERUM PROTEIN MAY AID UNDERSTANDING OF MULTIPLE MYELOMA

Studies of serum proteins provide important clues to the characteristics of cancer. Dr. John L. Fahey, General Medicine Branch, has reported the isolation of an abnormal protein from the serum of mice bearing plasma cell tumors. Analysis of the tumor tissue also yielded the abnormal protein, and there was a direct relationship between the amount of tumor and abnormal serum protein. The protein has been purified in preparation for further study, which it is hoped will lead to a better understanding of multiple myeloma in man.

STUDY CAUSE OF INCREASE IN BLOOD PLASMA PROTEIN ACCOMPANYING TUMOR GROWTH

It has been established that the growth of implantable tumors in mice is accompanied by a substantial increase in the blood plasma protein, alpha-globulin. Dr. Peter Bernfield, Tufts University School of Medicine, has reported a study undertaken to determine whether the increase of alpha-globulin during tumor growth is due to the appearance of new plasma proteins or to the increase in concentration of one of the normal plasma proteins. Alpha-globulin preparations obtained from normal and cancer-bearing mice were injected into rabbits. This caused a reaction in the blood serum of rabbits, in which a compound antagonistic to the mouse protein was developed. Chemical studies of this antiserum indicated that new plasma proteins with the characteristics of alpha-globulin appeared during tumor growth, while some proteins of the normal alpha-globulin fraction disappeared.

DIAGNOSIS OF CANCER

The ultimate goal of cancer research is the control of cancer in man. Attainment of this goal will be aided by the results of research on the development of diagnostic procedures which will enable the physician to identify cancer in its earliest and most curable stages.

ADVANCED UTERINE CANCER MAY BE ASYMPTOMATIC TWO TO THREE YEARS

The cytologic test for cancer involves the microscopic examination of a smear containing cells shed from internal organs and collected from various body openings. This procedure was developed by Papanicolaou, and modifications of it have been devised as aids in the diagnosis of cancer of the uterus and other parts of the body.

The first application of the test as a mass screening procedure for the detection of early uterine (womb) cancer in a large population of women was undertaken in Memphis and surrounding Shelby County, Tennessee, about five years ago. The project is a joint effort of the University of Tennessee and the Field Investigations and Demonstrations Branch of the National Cancer Institute with the cooperation of local medical and health groups. Analyses of data obtained in the screening of 108,000 women were previously reported. The results clearly indicated the value of the cell-examination test as a method for the early detection of cancer.

An additional analysis of the data obtained in the screening of 83,000 women during the first $3\frac{1}{2}$ years of the Memphis project has now been reported by Dr. John E. Dunn, Field Investigations and Demonstrations Branch. The results show that invasive (advanced) uterine cervical cancer may be asymptomatic for 2 to 3 years after onset. Uterine cervical cancer can be detected cytologically during this symptom-free period. This finding reaffirms the usefulness of the cytologic technique for the detection of unsuspected uterine cervical cancer.

CYTOANALYZER SPEEDS CYTOLOGY TEST RESULTS

In the cytologic test for cancer, a few drops of body fluid containing cells are fixed on microscope slides, which are then sent to a laboratory for examination. Each slide must be carefully studied by highly trained technicians in order to detect any malignant cells which may be present.

In order to speed the examination of cells and to pave the way for application of the uterine cervical cytologic test among large groups of women, an electronic device, the Cytoanalyzer, is being developed. A model of the instrument is now undergoing test at the Memphis cytology project. When this machine is perfected it will instantly detect abnormal, suspicious cells on the basis of certain structural characteristics. It will thus make possible the screening of hundreds of thousands of microscope slides daily and permit the extensive application of cytology as a cancer case-finding procedure.

STOMACH ACID SCREENING USEFUL IN ASYMPTOMATIC GASTRIC CANCER DETECTION

Scientists have suspected for a long time that stomach cancer is associated with a low stomach acid content. Dr. Claude R. Hitchcock, University of Minnesota Medical School, has found that screening for achlorhydria (absence of stomach acid) and hypochlorhydria (low stomach acid content) is an effective means of diagnosing a reasonable number of gastric cancers in the asymptomatic phase of the disease. During a 10 1/2-year period, over 12,000 men and women have undergone a screening test to detect those persons likely to develop stomach cancer. The portion of the total population that must be examined periodically because of increased likelihood of developing stomach cancer has been reduced to 6 1/2 percent. It is believed that population screening for gastric cancer becomes economically feasible through this method.

A much higher correlation of severe gastric mucosal atrophy was also found in persons with gastric cancer than in any other group except pernicious anemia patients. These findings suggest that achlorhydria and atrophic gastritis are precursors of gastric cancer. In the achlorhydric-hypochlorhydric group, the incidence of gastric cancer was 4.5 times greater, and in the pernicious anemia group it was 21.9 times greater, than in a comparable segment of the normal population. Dr. Hitchcock concludes that his studies suggest practical and effective criteria for determining which segments of the total population should be examined periodically, thus reducing to a practical size the group to be followed in the survey. Members of this group probably should be examined according to the following schedule, Dr. Hitchcock suggests: 1) pernicious anemia patients--gastrointestinal X-rays at 6-month intervals; 2) achlorhydric-hypochlorhydric persons--gastrointestinal X-rays at 9- to 12-month intervals.

250-KV X-RAY SUITABLE TO USE IN DIAGNOSIS OF CHEST CANCERS

Although encouraging results have been obtained in initial studies of supervoltage (1- to 2-Million electron volts) X-ray machines in the diagnosis of lung cancer, the short supply and high cost of the instruments have prompted a study of the possible use of lower powered X-ray machines for this purpose. Dr. John H. Harris, Jr., a National Cancer Institute Fellow in Radiology at the University of Pennsylvania, has reported on preliminary studies with 23 patients. The results indicated that diagnostic X-ray photographs made with a 250-kv (kilovolt) X-ray machine are strikingly similar in appearance to supervoltage roentgenograms and are useful for the demonstration of several lesions in the chest that are obscured by overlying bone in conventional studies. Thus, it appears that 250-kv roentgenography is a useful and generally available substitute for supervoltage diagnostic X-ray.

TREATMENT OF CANCER

Surgery and radiation continue to be the most effective means of treating cancer. Research in these procedures is designed to refine and improve them in order to achieve ever increasing prolongation of the lives of cancer patients.

LIVER CANCER OPERATION APPARENTLY SUCCESSFUL

Dr. George T. Pack, Sloan-Kettering Institute for Cancer Research, has reported an apparently successful operation for cancer of the liver, the one organ in the body capable of regrowth and replacement of tissue.

The patient was a 14-year old girl with a cancer of the right lobe of the liver, which was considered inoperable at first because of the friable nature of the tumor and heavy infiltration of the mass with blood vessels. The tumor was heavily irradiated, resulting in a 50-percent reduction in its size and an increase in firmness of both the tumor and surrounding liver tissue. The affected liver lobe was successfully removed and the patient was discharged 19 days later in good general physical condition.

PARTIAL BLADDER REMOVAL EFFECTIVE IN ADVANCED CANCER OF THE BLADDER

Partial removal of the bladder has been found effective in treating patients with advanced bladder cancer. Dr. Roger Baker, Georgetown University Medical School, has reported that this operation was performed upon 22 patients. At the time the report was written, the patients had been followed for three years or less. None had died and only two had recurrent cancer. In both these cases, the primary lesion had advanced through the bladder wall prior to surgery.

Chemotherapy has become established as a valuable adjunct to surgery and radiation in the past decade because of its effectiveness in temporarily halting the progress of certain cancers and in increasing the well-being of cancer patients. The hope of many scientists is eventually to find compounds or drugs that will selectively seek out and destroy cancer cells and tissues without harming the surrounding normal, healthy cells. Research toward this goal is proceeding simultaneously along several lines. These include, for example, clinical trials of compounds that have shown activity against animal cancers, screening of compounds in experimental animals for

antitumor activity, study of the effects of combination therapy--that is, two or more drugs used together or drugs used with surgery and/or radiation--and evaluation of known antitumor drugs in terms of dosage and toxicity.

CONTINUED SUCCESS IN CHEMOTHERAPY OF CHORIOCARCINOMA

Scientists of the Endocrinology Branch have continued to achieve encouraging results in the treatment of the highly malignant tumor of the uterus, choriocarcinoma, with the drug, methotrexate, in a newly devised intensive dosage regimen. The cancer is a rare type arising during or after preganacy from the organ that would normally develop into the after-birth, or placenta. In women with choriocarcinoma, this organ has become a malignant tumor that grows rapidly through the uterus and then spreads to the lungs and brain. It usually kills the patient in less than one year.

The tumor produces a hormone, chorionic gonadotropin, which is the same hormone responsible for positive pregnancy tests. This hormone is excreted in the urine of choriocarcinoma patients in huge amounts, which are markedly reduced as the patients improve under methotrexate treatment. Hence, the quantity of hormone provides a means of measuring the progress of treatment. Measurements of visible and palpable tumor masses and X-rays are also used as indices of tumor response to treatment.

Dr. Min C. Li, Donald B. Spencer, Dr. Roy Hertz, and Dr. Herbert A. Lubs, reported early in the year on results obtained in the treatment of four women patients. Three of them showed apparently complete suppression of cancer and disappearance of metastases for 12, 13, and 17 months, respectively. The fourth woman had been recently treated and her cancer similarly regressed.

According to a later report by Dr. Roy Hertz, the number of women treated has increased to 15, ten of whom are now restored to normal living and have been entirely free of symptoms for periods ranging from three months to two years. Three are still under treatment and two died during early phases of attempted treatment. Because of the marked variability in the spontaneous clinical course of such cancers, evaluation of therapy is difficult. However, the data constitute unequivocal evidence that they are benefitted by the drug used.

SEVERAL TYPES OF CANCER RESPOND TO TREATMENT WITH 6-AZAURACIL

Promising results from the use of a new drug for the treatment of cancer in human patients were reported by Dr. B. I. Shnider, General Medicine Branch. The drug, 6-Azauracil, belongs to a class of compounds known as antimetabolites, which function by interfering with the metabolism of cancer cells--the process by which they replenish the materials essential for their growth and reproduction.

The drug was given to a group of 44 patients, including adults and children, who were afflicted with various types of cancer, including acute leukemia and cancer of the gastrointestinal tract, lung, oral cavity, and breast. The objective was to determine whether 6-Azauracil had anticancer activity in human patients and if it could be tolerated by the body. Important therapeutic effects were noted in the acute leukemia cases, most of whom were children; one-third of the acute leukemia patients showed temporary improvement. Among the patients suffering from other types of cancer, two showed a decrease in size of metastatic lesions.

SULFUR MUSTARD TESTED AGAINST ADVANCED CANCERS

In a study reported by Dr. Nicholas A. Petrakis, University of California School of Medicine, a compound called SM-1 has been tested in clinical trials involving 54 patients with various types of advanced cancer. The drug is a sulfur mustard compound, related chemically to nitrogen mustard, and has the formula, 1,2-bis(beta-chloroethylthio)ethane. It was found effective in 8 patients with Hodgkin's disease and lymphosarcoma and in several patients with lung and ovarian cancer. The drug was administered intravenously and produced toxicity closely resembling that caused by nitrogen mustard. The bone marrow depression observed in all patients persisted three to four weeks and limited the use of the drug to monthly intervals. The therapeutic benefit and ease of administration of SM-1 appear of sufficient value to warrant expansion of the study of this agent.

NEW ESTROGENIC COMPOUND, MYTATRIENEDIOL, IS USEFUL IN TREATING HUMAN CANCERS

A newly synthesized drug, Mytatrienediol, has been found to be effective against several types of human cancer, including multiple myeloma and prostatic cancer. In a study reported by Dr. Daniel Laszlo, Montefiore Hospital, New York City, several patients with prostatic cancer showed clinical improvement and

a marked decrease to normal levels in the amount of an enzyme known as acid phosphatase. This enzyme is found in abnormally high amounts in prostatic cancer patients. A multiple myeloma patient who had been bedridden for six months became ambulatory on the 10th day of treatment.

Mytatrienediol is related to the female sex hormones. It is one of a number of estrogenic compounds with more than one physiological function that are being investigated for anticancer activity.

NEW ADRENAL STEROIDS PRODUCE REMISSIONS IN LEUKEMIA PATIENTS

Dr. Alfred Gellhorn, Columbia University, has reported that the administration of large doses of prednisone or prednisolone in 18 cases of acute leukemia resulted in clear-cut, complete remissions in 5 adults and partial remissions in 6 adults and children. Prednisone and prednisolone are newly synthesized adrenal steroids and are chemically related to cortisone, which is produced by the adrenal glands. Cortisone has produced a reasonably predictable remission rate in acute leukemia of children. However, in acute leukemia of adults, improvement has been noted only during the period of treatment with cortisone, and upon discontinuation of the drug, prompt intensification of the signs and symptoms of the disease usually occurs.

CHRONIC LEUKEMIA PATIENTS BENEFIT BY PROMPT ACTION OF NEW AGENT, CB-2348

The drug, Myleran (1,4-dimethanesulfonoxybutane) is an effective agent for palliative treatment of the blood cancer, chronic granulocytic leukemia. Its slow action is a disadvantage in critical situations in which rapid response is necessary. Dr. H. R. Bierman, City of Hope Medical Center, Duarte, California, has reported encouraging results from the use of a related compound, CB-2348, (1,4-dimethanesulfonoxy-1,4-dimethylbutane). The prompt action of CB-2348 in several patients suggests that it may be an additional helpful agent in the management of chronic granulocytic leukemia.

DON AND DON PLUS 6-MP CURB BONE CANCER GROWTH

Dr. W. P. Laird Myers, Sloan-Kettering Institute for Cancer Research, has reported on results of clinical trials with DON (6-diazo-5-oxo-L-norleucine), an antibiotic isolated from culture filtrates of a fungus, Streptomyces. Eleven patients with bone cancer were given DON; four of them were also

given the drug, 6-mercaptopurine, an antimetabolite. Evaluation of response to treatment included measurement of calcium in the blood and urine. Calcium is an element necessary for bone formation and is present in large quantities in the blood of patients whose bone is being destroyed by cancer. In this study, blood serum calcium levels returned to normal in eight cases and urinary calcium excretion decreased in nine cases. Dr. Myers suggests that DON and DON plus 6-mercaptopurine decreased the rate of bone destruction by the cancer.

COLCEMIDE, A PLANT DRUG, HAS BEEN FOUND EFFECTIVE AGAINST CHRONIC LEUKEMIA

Use of a drug derived from a plant, the common autumn crocus, has been reported by Dr. Pearl B. Holly, George Washington University School of Medicine. Tests with 60 patients over a period of three years indicate that the drug, Colcemide, (desacetylmethylcolchicine) produced favorable responses in six patients with chronic myeloid leukemia, a cancer of the bone marrow, and that it appears, therefore, to be a useful addition to the group of compounds effective in the treatment of this type of cancer.

TREATMENT OF LEUKEMIA IN ADULTS IS REVIEWED

In review of clinical research, Dr. Rose R. Ellison, Sloan-Kettering Institute for Cancer Research, has reported on the methods of managing acute leukemia in adults.

Both "specific" and supportive measures are used. "Specific" antileukemic therapy, which produces temporary improvement of the blood condition and consequent clinical well-being, makes use of the following agents: antimetabolites, including purine antagonists; folic acid antagonists, and Azaserine; ACTH and adrenocortical steroids, including cortisone, hydrocortisone, and prednisone; and localized irradiation. 6-Mercaptopurine and 6-chloropurine are used most extensively. In some instances, patients who become resistant to 6-MP are given methotrexate and unexpectedly develop complete or partial remissions. A larger group of patients is being treated by this method in order to confirm these observations.

Supportive therapy is an adjunct to antileukemic therapy and is important in the management of complications, such as bleeding, anemia, and low resistance to infection. Transfusions and antibiotics are used a supportive treatment.

The median survival time of a group of adults treated at

Memorial Hospital, New York City, was 6 months.

The incidence of all types of leukemia is increasing, and an estimated 11,000 new cases of leukemia are reported in the United States each year. In 1954, 10,443 deaths from this cause were certified.

SURVEY TREATMENT OF CHILDHOOD LEUKEMIA

In another review, Dr. C. P. Rhoads, Sloan-Kettering Institute for Cancer Research, has reported on methods of managing acute leukemia in children, using three main classes of agents. One consists of folic acid antagonists, such as methotrexate and aminopterin, which put the body in a state of relatively mild vitamin deficiency, not enough to damage normal cells but damaging to leukemia cells. Good partial remissions may be expected in 30 to 50 percent of the children, and complete remissions in 20 to 30 percent for many months.

The hormones that are related to the function of the adrenal glands constitute another group of agents. ACTH (secreted by the master endocrine gland, the pituitary, which is located in the brain) and cortisone have proved valuable in treating acute leukemia in children. Cortisone, which is more commonly used, produces good clinical remissions in 50 to 70 percent of the children treated. These remissions are generally of shorter duration than those produced by the folic acid antagonists, and resistance to hormones develops rapidly. There is apparently no cross-resistance between steroid hormones and folic acid antagonists.

The third group of anti-leukemic agents consists of purine antagonists--substances that interfere with the synthesis of purine in the cell. The antimetabolites, 6-mercaptopurine, thioguanine, and 6-chloropurine are typical purine antagonists. In a series of 87 children, 47 percent had good clinical remissions with 6-mercaptopurine and 18 percent had partial remissions. A patient resistant to one of these purine antagonists is resistant to all, but there is no cross-resistance between these compounds and folic acid antagonists or steroids. An antibiotic, Azaserine, has also been found to be a purine antagonist. When given in combination with 6-mercaptopurine in selected cases, the development of resistance may be delayed. Three out of 29 patients started on this combination were still in remission at the time of reporting after more than 12, 17, and 21 months, respectively.

RESERPINE LENGTHENS LIVES OF MICE BEARING LEUKEMIA

In studies with experimental animals, Dr. Abraham Goldin, Laboratory of Chemical Pharmacology, has reported that a single treatment with the tranquilizing drug, reserpine, produced an almost three-fold increase in the survival time of mice with advanced leukemia. In the study, the mice were inoculated with a suspension of leukemic cells and were allowed to develop leukemia until the local tumor had reached a diameter of about 9 to 12 millimeters, at which time the disease was generally systemic as well as local. Treatment with reserpine inhibited local tumor growth and frequently resulted in complete disappearance of the local tumor at the site of inoculation. However, transplantation of spleen from several such mice resulted in leukemic growth, indicating that systemic infiltration had not been totally suppressed.

The mechanism by which reserpine exerts its antileukemic action is not known. Whether the antileukemic effect is direct or mediated through the host also is not clear. However, at high dose levels, reserpine-treated animals--both those with leukemia and normal controls--were severely depressed by the drug and failed to eat or drink for a week or longer. Although reserpine was not as effective as methotrexate, its importance lies in having made available for laboratory study a new group of antileukemic agents.

TRANQUILIZERS HALT GROWTH OF SOLID TUMORS IN EXPERIMENTAL ANIMALS

The observation that reserpine prolongs the survival time of leukemic mice and in many cases causes a reduction in the size of the local tumor mass, led to studies of the effects of tranquilizing drugs on animals bearing solid tumors (Sarcoma 37). Dr. Morris Belkin, Laboratory of Chemical Pharmacology, has reported that both reserpine and chlorpromazine placed the cancerous mice in a deeply tranquilized state, during which period they kept their eyes closed and burrowed their heads in the shavings. At the end of 5 to 6 days they were dead, presumably from lack of food and water and perhaps from some specific effect of the drugs.

In the animals treated with either drug, tumor growth ceased at once, whereas tumors in untreated control animals continued to grow at a normal rate and in 5 to 6 days attained nearly three times the size of tumors in the treated mice. Although it is difficult to assess cellular damage in advanced tumors because of spontaneous necrosis, histologic examination appeared to show more cellular damage in the treated than in the control

mice. However, it is not clear whether the tranquilizing drugs are directly carcinolytic or whether their anticancer action is mediated through the host.

STUDY THERAPEUTIC EFFECTS OF NEW ANTICANCER AGENTS IN LABORATORY ANIMALS

Dr. Erich Hirschberg, Columbia University College of Physicians and Surgeons, has reported the development of an anticancer agent, benzimidazole mustard, 2-[di-(2-cloroethyl)aminomethyl] benzimidazole hydrochloride, which is effective against several types of cancer in experimental animals. This compound is related to nitrogen mustard but is less toxic. In therapeutic effectiveness, this compound compares favorably with Myleran, chlorambucil, and several mustard derivatives. The mechanism of action of benzimidazole mustard is comparable with that of nitrogen mustard, which is a cell poison rather than an antimetabolite. The toxic side effects, measured in rabbits and dogs, are primarily on bone marrow function.

Dr. Roland K. Robins, New Mexico Highlands University, reported earlier that a new compound, 4-aminopyrazolo(3,4-d)pyrmidine, significantly inhibited several types of cancer in mice. In a report co-authored with Dr. Howard E. Skipper, Southern Research Institute, he now reports that a number of chemically related compounds possess the ability to inhibit the growth of an experimental cancer, Adenocarcinoma 755, in mice. Consistent and significant activity was produced by the 4-amino-, 4-monoalkylamino-, and 4-dialkylaminopyrazolo(3,4-d)pyrimidines, and the 1-alkyl derivatives of these compounds.

Development of a new class of tumor-inhibitory compounds, 5-fluoro pyrimidines, has been reported by Dr. Charles Heidelberger, University of Wisconsin Medical School. 5-Fluorouracil and 5-fluoro-orotic acid had appreciable tumor-inhibitory activity against a variety of transplanted rat and mouse tumors. In the Ehrlich ascites carcinoma, for example, 5-fluorouracil produced a 420 percent increase in survival time. Several animals survived 100 days, at which time they were tumor-free, as judged by gross examination and attempted transplantation experiments.

ZYMOSAN, A YEAST PRODUCT, INCREASES RECOVERIES IN TUMOR-BEARING MICE

Dr. William T. Bradner, Sloan-Kettering Institute for Cancer Research, has reported that injection of a single low dose of the yeast product, zymosan, into the body cavity of tumor-

bearing Swiss mice significantly increased recovery of the mice. After about 2 weeks, 60 percent of the tumors in treated animals were destroyed and were completely extruded, as compared with 6 percent of tumors in controls. Injection of a very large dose had significantly less effect; only 28 percent of the animals lost their tumors. Zymosan tested against the same tumor grown in tissue culture had no direct effect on the cancer cells. It is concluded that a host-mediated phenomenon appears to be responsible for the loss of tumors by zymosan-treated animals.

CONTINUOUS THERAPY IS BEST FOR LEUKEMIC MICE

The formulation of correct dosage schedules remains one of the chief problems in cancer chemotherapy. A series of studies is being made by scientists of the Laboratory of Chemical Pharmacology and the Biometry Branch to develop optimum treatment schedules in the management of mouse leukemia with methotrexate. One project was conducted to determine the influence of the duration of treatment of advanced mouse leukemia with methotrexate, since an earlier study had suggested that failure to achieve cures was due principally to the chronic toxic effects of the drug resulting from prolonged treatment. John M. Venditti, Laboratory of Chemical Pharmacology, has now reported that it is not advantageous to discontinue the daily injections in order to allow the host animal to recover from the toxic effects of the drug.

In the study, treatment of the test mice was started on the eighth day following inoculation with leukemia. Control mice, which received no drug treatment, survived an average of 2.2 days longer, or 10.2 days. Treated mice survived at least 12 days longer, or a total of 20 days after inoculation. However, when daily treatment was discontinued, the mice survived only two to three days. These results suggest that the drug, as given under this dosage schedule, did not produce sufficiently extensive regression of advanced tumors to permit discontinuation of treatment in an effort to control toxicity.

COMBINED DOSE SCHEDULE HELD SUPERIOR IN TREATING LEUKEMIA IN CHILDREN

As a result of a study of the effects of 6-mercaptopurine in the treatment of acute leukemia in children, Dr. Carol B. Hyman, University of Southern California, suggests that an initially high dose rate followed by a smaller dose rate would produce the best therapeutic results. The literature indicates that 6.6 milligrams of the drug per kilogram of body weight approaches the maximum daily dose that can be taken by the

majority of patients without serious toxic reactions. Dr. Hyman has now reported that this dose rate reduces the interval between the start of therapy and the onset of remission and that serious toxic reactions do not appear during the first two weeks of therapy on this schedule. A maintenance dose of 2.2 mg per kg per day of the drug produces longer remissions, Dr. Hyman reports. Hence, a combined dosage schedule would seem to be advantageous because of the shortened period preceding onset of remission and the benefits of continuous therapy.

DRUGS SENSITIZE TUMOR; INCREASE EFFECTIVENESS OF RADIATION THERAPY

In studies of combination therapy, experiments on the treatment of cancer in mice show that sensitization of a tumor by anticancer drugs augments the effectiveness of radiation therapy. Dr. Morton Kligerman, College of Physicians and Surgeons, Columbia University, has reported that mammary tumors of mice treated by both chemical and radiation therapy exhibited more therapeutic response than did those treated by X-rays or chemicals alone. The effect of therapy was measured on the basis of percentage of tumors showing regression, degree of regression, "cure rate," and average weight of tumors at the end of a one-month observation period. These findings suggest that the limit of curability of cancer by radiation therapy is no longer determined by a lack of sufficiently powerful radiation sources, and that tumor sensitization by biochemical means appears to be an important method of increasing the response of tumor cells as compared with that of normal tissue.

STUDY THERAPEUTIC EFFECT OF MULTI-DRUG TREATMENT

On the assumption that quantitative biochemical differences between tumor and host may serve as a basis for cancer chemotherapy, investigators of the College of Physicians and Surgeons, Columbia University, are studying the use of multi-drug therapy to exploit such differences to a point at which maximum anticancer effect and minimum toxic effect against the host are achieved.

Dr. Daniel M. Shapiro has reported on results of tests using 6-aminonicotinamide (6-AN), which exerts strong antitumor activity against mammary Adenocarcinoma 755 in mice. Tumor regression was accompanied by marked weight loss at high dose levels and less weight loss at low dose levels. Combination of the drug at low dose levels with 8-azaguanine resulted in tumor regression without appreciable weight loss in the host. Addition of testosterone (male sex hormone) to this double combination further reduced weight loss without changing the

degree of tumor regression. The fact that 6-AN is a potent antagonist of niacin (one of the B-vitamins) emphasizes the potentialities of vitamin antagonists and suggests that niacin antagonists be investigated as a source of anticancer agents.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

HEART DISEASE

1957

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Heart Institute

When medical science advances into a new frontier of disease, its earliest research findings there establish solitary outposts of knowledge separated by a wilderness of ignorance. Each additional research finding clears away a little more of the ignorance and the isolated findings can be seen in increasingly meaningful relationships with one another.

As the individual outposts of knowledge gradually grow and converge, understanding of the disease begins to develop. Then, as continuing research explores remaining unknowns, joining more of the individual areas of scientific understanding, there finally comes specific knowledge that can be applied in the control of the disease.

The progress made in research on diseases of the heart and blood vessels has been considerable. Some types of cardiovascular disease can be prevented, some cured, and the understanding of others has been increased. To this progress the research conducted by the National Heart Institute, and that supported in universities and hospitals throughout the country by its research grants, have contributed much. In 1957, investigations on baffling cardiovascular problems, including major diseases such as arteriosclerosis and hypertension, were productive and new knowledge was gained in many areas. Some of the individual findings are described below.

Prepared, January 1958

ATHEROSCLEROSIS AND CORONARY HEART DISEASE

DETAILED STUDIES IN HUMANS CONFIRM THAT SOME DIETARY FATS LOWER SERUM LIPIDS

That some dietary fats produce higher serum cholesterol levels than others has been shown conclusively by Dr. Edward H. Ahrens, an NHI grantee, and his co-workers at the Rockefeller Institute for Medical Research. They have found in experiments in humans that the highest levels of cholesterol result when butter and coconut oil are eaten as the sole dietary fats. Intermediate levels are produced by palm oil, lard, cocoa butter, and olive oil. The lowest levels are produced by peanut, corn, cottonseed, and safflower oils.

In a summary of their detailed metabolic studies published in the Journal of the American Medical Association, Dr. Ahrens' group also reports a direct correlation between degree of saturation of the dietary fat and the levels of cholesterol and phospholipids in the circulation. For example, removal from corn oil of eighty percent of the minor components such as sitosterol, carotenes, and tocopherols failed to abolish its cholesterol-lowering effects. On the other hand, saturating corn oil by subjecting it to a hydrogenating process, of the sort used commercially to "harden" fluid vegetable oils, caused it to raise human serum cholesterol levels.

Exploring the effects of vegetarianism, the scientists removed from the normal diets of a group of outpatients all meats, poultry, fish and dairy products, except washed cottage cheese and skim milk. Margarine was substituted for butter. They found that in all cases the serum cholesterol levels were lower on the vegetarian regimen. When the patients were given their nourishment as liquid homogenates containing corn oil at 40% of calories, their cholesterol levels were still lower.

With regard to the preventive implications of the findings, the investigators state: "In view of the fact that it has not been demonstrated in man that lowered levels of serum lipids will alter his susceptibility to atherosclerosis and in view of the many questions that remain unanswered in this year 1957, it seems inadvisable at this time to recommend that the general public eat more or less fat or avoid fat of any particular type. We believe that much more basic information should be gathered before widespread changes in dietary habits are adopted. It is entirely probable that this period of indecision will be short-lived, since progress is rapidly being made in fundamental areas of lipid biochemistry and nutrition."

"On the other hand, where the likelihood of death or disability from atherosclerosis is great--in patients with a personal or family history of arteriosclerosis and in those with

hypercholesteremia--it is reasonable to act on an unproved hypothesis if the risk of doing so is small."

DOG STUDIES EMPHASIZE ROLE OF POTASSIUM IN DEATH FROM CORONARY OCCLUSION

Physicians at the Medical College of Georgia have found that they can prolong life of dogs following coronary occlusion by giving them infusions of either insulin-glucose or sodium bicarbonate, which reduce the release of potassium from the injured heart muscle.

The investigators, Drs. Abraham Cherbakoff, Seiichi Toyoma, and W. F. Hamilton, were prompted to try these potassium-inhibiting preparations by evidence from several sources suggesting that release of potassium from damaged heart muscle may excite abnormal heartbeats ("extrasystoles") that could interrupt the normal rhythm and cause many of the deaths that occur during and following coronary heart attacks.

Their studies, which are reported in the journal Circulation Research, are a product of a cardiovascular research training program supported in part by NHI. The results of these studies support the theory implicating myocardial potassium release in the incidence of abnormal heart rhythms following coronary occlusion. The investigators are thus convinced that the association of rising levels of potassium in blood draining the heart muscle with loss of normal heartbeat will ultimately play an important role in medical understanding of the fatal outcome of coronary occlusion.

The investigators report first measuring normal levels of potassium in the blood draining the heart muscle in 25 dogs by threading catheters into the coronary sinus, the heart muscle's largest vein. Subsequently they knotted threads tightly around the left main coronary artery in all 25, while continually monitoring their electrocardiograms and drawing coronary sinus blood samples to measure potassium release.

In 16 of the 25 the investigators began to pump slowly either the insulin-glucose or the sodium bicarbonate solution (or a mixture of both) into a vein before occluding the coronary artery, and they continued these infusions through the tie-off operation. This group of 16 was the experimental group.

In 8 of the 9 "control" dogs that received no infusions, coronary occlusion caused a rise in coronary sinus potassium accompanied by bursts of extrasystoles, and a loss of heart rhythm within five minutes in 5 of the 8, the scientists reported. In the other 3, the potassium dropped toward normal again and the extrasystoles diminished, only to rise again, with death following

in 16 to 20 minutes after the occlusion. In the ninth control, the coronary sinus potassium did not go up, and there was no loss of heartbeat from the occlusion.

In 5 of the 16 in the experimental group, coronary sinus potassium rose despite the infusions and they died in heart rhythm failure just as the control animals did. But in the other 11, the potassiums stayed low and heart rhythm remained regular for an hour or more (6 animals) or for three hours until they were sacrificed.

HEART ATTACKS PRODUCED IN RATS BY SATURATED-FAT AND CHOLESTEROL DIET

A high incidence of heart attacks--coronary thrombosis with myocardial infarction--has been produced for the first time in significant numbers of experimental animals. This achievement, reported in the Journal of the American Medical Association, was made by NHI grantees Drs. W. Stanley Hartroft and Wilbur A. Thomas of the Washington University School of Medicine in St. Louis.

The scientists produced the heart attacks in white rats by feeding them diets high in saturated fats (butter, lard, or hydrogenated vegetable oils) and supplemented with cholesterol and other compounds which tend to raise the blood cholesterol (cholic acid and thiouracil). In animals fed such diets for fourteen weeks, extensive myocardial infarction was observed in incidences ranging from one animal in nine to as high as five of nine.

"We believe that these animals developed myocardial infarcts because factors that raised the levels of blood cholesterol and a high supplement of saturated fat (40% butter or lard) were combined in the same dietary regimen," the scientists explain. "In order to evaluate properly the relative importance of these factors, however, more experiments are needed; some of these are already underway in our laboratory. Now that we have produced what seems to be a significant incidence of the lesion, it should not be difficult to test the effects of various dietary situations along with other factors such as age, sex, and diabetes on the incidence of myocardial infarction in rats under these conditions. We hope by this approach to elucidate some of the interrelations between known atherogenic mechanisms and those actually responsible for the fatal complications of atheroma."

The investigators caution that no one yet knows whether the fatty components of the lesions that clog arteries are primarily responsible for human myocardial infarctions, or whether these fatty deposits are just secondary to factors still

unknown--for example, underlying disturbances in the wall of the artery.

"Until specific answers to these and other questions are obtained," they point out, "it is likely that myocardial infarction will continue to be the most important single cause of death in the American population."

"SILENT" CORONARY ATTACKS ACCOUNT FOR ONE FOURTH OF ALL INFARCTS IN NHI STUDY

National Heart Institute scientists conducting a long-term epidemiological study at Framingham, Massachusetts, have found that "silent coronaries"--heart attacks that go unrecognized--accounted for one-fourth of the coronary attacks that occurred in the study population of Framingham.

Sixty-seven of the 5,209 individuals who comprise the study population developed coronary attacks (myocardial infarctions) as evidenced by the electrocardiograph. Nine percent of these denied any symptoms whatever, and an additional sixteen percent reported chest, abdominal, or other symptoms that were not interpreted by either the patient or his physician as coronary.

A summary of the findings, reported by Drs. Joseph Stokes, III, and Thomas R. Dawber of the Framingham Study, has appeared in the journal Circulation.

Comparing the characteristics of the "silent coronary" with those of the more typically painful attacks, the scientists found no significant differences regarding age, occupation, weight, blood pressure, or heart size. The heart damage from the silent coronary did, however, tend to differ in its anatomical location in the heart. Angina pectoris, the chronic chest pain of poor coronary circulation, did not result as often from the silent coronary, the study showed.

UNDERSCORE IMPORTANCE OF ESTROGENS IN IMMUNITY OF WOMEN TO ATHEROSCLEROSIS

The protection enjoyed by hens, as opposed to roosters, against diet-induced coronary artery disease is abolished when the ovaries of the hens are removed, investigators at Michael Reese Hospital in Chicago have found. The scientists, Drs. Ruth Pick, Jeremiah Stamler, and Louis N. Katz report this finding from their NHI grant-supported studies of cholesterol-fed chicks in the journal Circulation Research.

The comparative immunity of hens to atherosclerosis was established in earlier experiments at Michael Reese. When fed 1% cholesterol in their diet, young roosters soon develop large

fatty deposits in the arteries of their hearts. Hens are much more resistant; even diets with twice the cholesterol will not produce the coronary lesions in hens. The earlier studies also showed that the coronary deposits induced in the roosters by the 1% cholesterol diet would disappear when they were given female sex hormones.

The Michael Reese group reports that cholesterol-fed hens which were castrated at five weeks of age developed severe coronary atherosclerosis comparable to that of the roosters. With the loss of their estrogen-secreting ovaries, their immunity to coronary disease was also lost.

"The parallelism between man and chick in this regard is striking," they explain. Thus pathologic studies demonstrate that ovariectomy in young women--as in hens--markedly suppresses their usual resistance to coronary atherogenesis.

"These observations," they conclude, "constitute additional evidence that the remarkable immunity of premenopausal women to coronary atherosclerosis and coronary heart disease is in a major way a resultant of ovarian estrogenic secretion."

FURTHER INSIGHTS GAINED INTO CHEMICAL NATURE OF "CLEARING FACTOR"

Recent experiments by Dr. Edward Korn of the National Heart Institute have provided a further clue to the chemical identity of lipoprotein lipase, the "clearing factor" enzyme that breaks down large fatty particles (beta lipoproteins and chylomicrons) in the blood.

In the Journal of Biological Chemistry Dr. Korn has reported evidence indicating that lipoprotein lipase contains components that are closely related in chemical structure to heparin, a substance from mammalian liver used medically to prevent blood clots. Chemically, heparin is an acid carbohydrate called a "mucopolysaccharide".

Dr. Korn and his colleagues in the Laboratory of Cellular Physiology and Metabolism have obtained from bacterial cultures a preparation of enzymes which will break down, or degrade, heparin into its lesser chemical structural units. It has been found that the heparin-degrading bacterial preparation will also inactivate lipoprotein lipase. Furthermore, the experimental conditions under which it acts on heparin and lipoprotein lipase are identical for both substances.

"All the conditions which activate or inactivate the bacterial heparinase have the identical effect on the ability of this preparation to inactivate the lipoprotein lipase," he reports.

"This is interpreted as evidence for the presence of a heparin-like mucopolysaccharide as an integral part of the enzyme, lipoprotein lipase."

Heparin has been linked in a poorly understood relationship with lipoprotein lipase since the action of this enzyme on blood was first seen in 1948. At that time a scientist in another institution noticed that injections of heparin would "clear" from blood the large fatty particles (chylomicrons and beta lipoproteins) which give blood plasma a "creamy" appearance after eating a fatty meal.

The Heart Institute scientists subsequently found that this "clearing reaction" to heparin is due to the release in blood of lipoprotein lipase. They have been trying to purify this enzyme and learn its chemical identity because they feel it may be important in normal fat metabolism.

Dr. Korn and his colleagues obtained the bacterial heparinase preparation because they hoped to learn something about the chemical structure of heparin as well as that of lipoprotein lipase. Heparin, an anticoagulant with important research as well as clinical uses, has resisted chemical analysis since its discovery forty-one years ago. It still cannot be synthesized and must be processed at great cost from the livers of animals.

The scientists found the bacterium with the heparin-digesting enzymes in NIH topsoil. This organism, newly named Flavobacterium heparinum, can use heparin as a source of nourishment because it has these digestive enzymes.

With continued study and use of these bacterial enzymes in this connection, information on the chemical structure of both heparin and clearing factor may be gained which will help reveal the exact role of the clearing enzyme in the body.

VARIABILITY IN CHOLESTEROL LEVELS OF MEDICAL STUDENTS AT JOHNS HOPKINS

Dr. Caroline Bedell Thomas of the Johns Hopkins University has been engaged since 1946 in a study of the variability of the total serum cholesterol in 759 medical students--young healthy men and women between 19 and 33 years of age attending that University. The long-term objective of the study, which is being aided by NHI grant support, is to ascertain whether or not the cholesterol level early in life can be used as one of a number of measures to predict tendencies to atherosclerotic and hypertensive heart disease later in life.

In the Journal of Chronic Diseases, Dr. Thomas and Mrs. Frieda Eisenberg report that these healthy young adults vary greatly

not only in the level of their serum cholesterol (100 to 400 milligrams per 100 cubic centimeters with an average of 230) but also in their degree of cholesterol stability. Thirty subjects were reexamined at the end of four to eight years. "The great majority of those with initially low serum cholesterol levels showed relatively little variation," they state, "while those with higher cholesterol levels showed much greater variability. That this observation is important from the point of view of the individual is apparent when it is recalled that, on the average, higher cholesterol levels and greater cholesterol variability distinguish patients with coronary artery disease from control subjects."

Fifty-three, or six percent of the students were found to have definite hypercholesteremia (levels over 300). "Nothing unusual was found with regard to the health of these hypercholesteremic subjects themselves," the scientists report, "but a history of coronary artery disease affecting the fathers of these students occurred with unwarranted frequency." Nearly a third of these students (32.1 percent) had a parent affected by coronary disease, while only 11 percent of the rest had such a family history. Comparatively few of the students had levels below 150 and only three in the entire group had a level below 125 mg. per 100 cc.

FATTY MEALS MAY REDUCE CORONARY FLOW BY "CLUMPING" BLOOD

Fatty meals may contribute to the reduction of coronary blood flow by changing the consistency of the blood, it has been indicated in an NHI grant-supported study by Drs. A. V. Williams, A. C. Higginbotham, and M. H. Knisely of the Medical College of South Carolina. The findings were reported in the journal Angiology.

Ten patients believed susceptible to attacks of anginal pain were selected for study by the investigators from regular medical practice. Microscopic observations of the circulating blood and vessel walls were made on the patients' bulbar conjunctiva--delicate membranes that cover the eyeballs--under two conditions. The membranes were first studied following a period of fasting, usually overnight, and second, following a high-fat meal or a fat-enriched meal, in which each patient received between 90 and 100 grams of fat.

The investigators found that after the high-fat meals there was an immediate altering of the physical consistency of the blood, by agglutination, causing the blood to become more resistant to passage through the narrowest vessels. In 8 of the 10 patients, there was a conspicuous increase in blood clumping which could be easily observed in the eye membranes at 50 X

magnification. Three of the patients, those who later experienced anginal pain, had highly agglutinated blood and also had visibly plugged small vessels.

In a corollary experiment some, but not all, normal healthy medical students studied exhibited mild blood cell clumping following a fatty meal.

Discussing the effect of clumped blood cells on the hydraulic conditions of blood flow, the investigators point out that "in the presence of coronary atherosclerosis, it seems probable that increased clumping of the blood cells decreases the flow of blood through the already narrowed coronary vessels, causing cardiac ischemia, hypoxia, and coronary insufficiency. If the plugging of small coronary vessels with masses of sludge is sufficiently prolonged, that should contribute to myocardial infarction."

MECHANISM REGULATING BLOOD CHOLESTEROL REACTS TO BOTH INTERNAL AND FOOD SOURCES

With evidence accumulating that the amount of cholesterol in the blood is not governed by the amount eaten in the diet, there has been increasing research interest in the body mechanisms that hold blood cholesterol constant, regardless of intake, by regulating its manufacture in the liver.

Findings from animal experiments by Drs. Leon Swell, E. C. Trout, Henry Field, and C. R. Treadwell of the Veterans Administration Center, Martinsburg, West Virginia, and the George Washington University Medical School in Washington, D. C., emphasize the sensitive "automatic" nature of these mechanisms for holding blood cholesterol constant. The NHI grant-aided studies show that the mechanisms respond to cholesterol entering the blood, not only from digested food, but also from the body's own sources of manufacture and supply.

In operations on fasting rats, the investigators sidetracked one of the major supply lines through which cholesterol made in the liver is carried to the blood. This supply line is the thoracic duct, a large vessel that collects lymphatic fluid draining from the liver, intestines, and other abdominal organs and channels it through the chest into veins leading to the heart. The scientists directed its flow outside the body, thus "bleeding" the rats of a large proportion of their internally manufactured cholesterol.

This did not result in any lowering of blood cholesterol--the levels stayed the same. The rats were found to be balancing the loss by making new cholesterol and pouring it into the blood from the liver (through other routes than the thoracic duct).

This was shown by injecting the rats with radioactive acetate, which is picked up by the liver to make new cholesterol. The newly made cholesterol was thus "labeled" and could be distinguished with a Geiger counter from all other cholesterol in the rats. Eight times as much radioactivity was found in the blood of the cholesterol-bled rats after acetate injections as in another fasting group of normal rats.

The study raises the possibility that the entry into the blood of cholesterol from digested food by way of the thoracic duct may be a major factor in controlling the rate it is made in the liver, the scientists explain. In the light of their findings, the investigators also question whether reducing cholesterol in the diet is effective in lowering it in blood or tissues.

CHOLINE DEFICIENCY FOUND TO PREVENT HIGH FAT LEVELS IN BLOOD OF RATS

The role of choline in the development of hyperlipemia, or "fatty" blood--a condition thought by most authorities to predispose to atherosclerosis--has been further clarified by recent research findings of Drs. George F. Wilgram and Charles H. Best of the University of Toronto and Dr. Lena A. Lewis of the Cleveland Clinic Foundation. Part of the study was supported by an NHI grant.

The addition of cholesterol to diets containing adequate choline, a chemical found in both plants and animals, has been reported to lead to a high level of blood fats and cholesterol in rats. The new findings show that this hyperlipemia and hypercholesterolemia can be prevented by a deficiency of choline.

These investigators report in the journal Circulation Research that blood lipids were markedly reduced in a group of choline-deficient rats as compared with a control group that received adequate choline. Dietary cholesterol was found to lead to hyperlipemia and hypercholesterolemia only in the presence of dietary choline. Furthermore, the addition of cholesterol to choline-deficient diets did not result in an elevation of blood lipids or blood cholesterol, indicating a close metabolic interdependence between choline and cholesterol.

"It would appear from our results that adequate dietary choline is one of the many requirements to make hyperlipemia and hypercholesterolemia possible," the researchers state.

MARGARINE AND BUTTER SEEN TO AGGRAVATE EXPERIMENTAL ARTERY DISEASE IN RABBITS

The feeding of either butter or margarine has been found to aggravate experimental arteriosclerosis produced in rabbits by injections of tiny blood clots. Feeding of corn oil, however, was found to have no effect on the disease.

The findings made in NHI grant-supported studies by Drs. W. A. Thomas, N. Konikov, R. M. O'Neal, and T. K. Lee of the Washington University School of Medicine in St. Louis, were reported in the A.M.A. Archives of Pathology.

The arteriosclerosis, which developed during six weeks of injecting homogenized clotted rabbit blood, formed in the lung arteries of the rabbits, apparently at the sites where the injected blood clots had lodged. The lesions consisted of fibrous thickening and fatty deposits in the artery wall, as does human atherosclerosis.

Eighty percent of 25 clot-injected rabbits fed butter by stomach tube developed "moderate or severe" lung artery lesions. Seventy-five percent of a margarine-fed group developed a comparable degree of lung arteriosclerosis. Only 40 percent, however, of a corn-oil fed group developed "moderate or severe" lung lesions--the same percentage as that of a control group given plain sugar water with their clot injections.

The St. Louis scientists caution that their findings justify no firm conclusions--only "thoughtful speculation"--about the role of dietary fats in atherosclerosis as it occurs in man. Many authorities believe that clot formation is important only in the later phases of the widespread form of the human disease--that the fatty deposits come first. It is not definitely known which comes first though and the St. Louis scientists point out that "If atherosclerosis is basically a mechanistic affair resulting from the deposition of thrombi (clots) on the vessel wall or from hemorrhage within, then appropriate fatty meals might affect the disease in the same way throughout its course."

"At least in one specific situation," they conclude, "butter and oleomargarine (highly saturated fats) have a profound biological effect whereas corn oil (a relatively unsaturated fat) has no demonstrable biological effect."

ARTERY PIECES IMPLANTED IN RABBIT EYE ENABLE STUDY OF ATHEROSCLEROSIS

Experimental atherosclerosis can be studied under direct vision in the lining of arteries by displaying pieces of artery in the transparent front chamber of the rabbit eye. This method--used in the past for tumor research--was introduced for

arterial research by Dr. A. C. Higginbotham at the Medical College of South Carolina, an NHI grantee, now of the School of Medicine, West Virginia University.

Dr. Higginbotham removes from a donor animal square pieces of either healthy arteries or arteries with uniform atherosclerosis. A tiny slit is then made in the extreme edge of the cornea of a host rabbit's eye and an artery piece is inserted and slid across, in front of the iris, to be wedged between iris and cornea on the opposite side of the eye. Another piece of artery from the same donor, as identical as possible to the implanted one, is kept to be studied for later comparison with the implant.

Recovery is prompt and uncomplicated, according to Dr. Higginbotham. Connective tissue grows to attach the transplant to the host's iris within eight days. Within thirty days blood vessels can be clearly seen to grow from the iris and invade the artery piece's outer layer (adventitia). The thickened and roughened inner lining (intima) of a diseased artery piece also grows a rich supply of blood vessels, but that of a healthy artery piece does not.

Transplants of aorta--normal and atherosclerotic--had been in place for between six and seven months in the eyes of Dr. Higginbotham's animals at the time of his report in the journal Science. "Apparently there will be time", he states, "to permit long-term studies of the effects of drugs, diets, and other experimental regimens on the structure and blood supply of the transplants."

DIET-INDUCED TENDENCY TO ARTERIOSCLEROSIS SLOWED IN ROOSTERS BY EXERCISE

Researchers at Howard University have found in NHI grant-supported studies that exercise will slow the development of experimental atherosclerosis produced in young roosters by a high-fat, high-cholesterol diet.

Seven weeks on a diet of mash enriched with two percent cholesterol and five percent cottonseed oil raised blood cholesterol in a group of cockerels considerably above those of a control group fed a normal diet in experiments by Drs. H. W. C. Wong, M. B. Anderson, J. K. Kim, D. J. Liu, and E. W. Hawthorne. Thus avian atherosclerosis--a form of experimental arteriosclerosis widely studied for clues to the nature of its "counterpart" in human arteries--was produced in these roosters in the form of yellow "plaques", or fatty deposits, lining the largest of their arteries.

The benefits of exercise were seen when this group was compared with another which was treated identically, except they were exercised twice daily during the seven weeks of cholesterol feeding. This group, the investigators found, had markedly lower blood cholesterol levels. "In addition," they report, "there was a subsequent reduction in the formation of atheromatous plaques on the abdominal aorta and coronary arteries of the exercised birds when compared with the cholesterol-fed only."

DIETS BOTH HIGH AND LOW IN FAT PRODUCE SAME INCIDENCE OF CORONARY LESIONS IN RAT

Experiments in rat coronary disease at the University of Chicago show that the incidence of atherosclerotic deposits produced in the coronary arteries of rats by a diet of lard and casein (milk protein) does not depend on a high level of either fat or cholesterol in the diet. The same incidence of coronary deposits was found in rats fed a diet of 10% lard as in those fed 30% lard.

Reporting in the A.M.A. Archives of Pathology on their studies, which were NHI grant-supported, Drs. Richard Jones, Robert Wissler, and Sheldon Huffman state that this finding was unexpected for a high level of either fat or cholesterol is generally believed to be a key factor in producing coronary atherosclerosis in animals and, many investigators believe, in humans as well.

The failure of earlier attempts to produce the disease in rats led the Chicago group to try the synthetic diet, in which the ingredients could be systematically varied. Most of the earlier approaches involved adding quantities of cholesterol or animal fat to an otherwise balanced stock diet. This often produced atherosclerosis in rabbits and chickens, but it generally failed in the more resistant rat. The synthetic diet allowed regulation of other constituents and the introduction of deficiencies and imbalances impossible with a stock diet.

The lard-casein diet worked well, the Chicago group reported. It produced a type of coronary disease closely resembling human coronary atherosclerosis. The unexpected finding that widely different levels of fat intake did not influence the incidence of coronary lesions suggests that other factors in the diet than level of fat intake are important in producing the coronary disease. Continuing research with the use of this diet may disclose what they are and possibly point up some additional dietary factors bearing on human atherosclerosis.

MENSTRUAL CYCLES OF WOMEN ASSOCIATED WITH HEALTHY CORONARY ARTERIES

Of sixteen women under 53 years of age who died of cornary heart attacks (myocardial infarction) in a St. Louis hospital, a study by physicians of their autopsy records revealed that only two had not previously undergone menopause or "change of life".

The investigators, Drs. Robert S. Spitzer, Kyu Taik Lee, and Wilbur A. Thomas of the Washington University School of Medicine, conducted the NHI grant-aided study of menopause and fatal heart attack in young women to learn more about the relationship between sex hormones and heart attacks. Though common in young men, heart attacks rarely strike women during their childbearing years, presumably because the sex hormones produced by their ovaries have a protective effect.

The St. Louis study seems to confirm this widely held belief. Of the sixteen young coronary victims, the two who had not stopped menstruating had been suffering from other disorders with which coronary disease is often associated. One, who died at age 35, had glomerulonephritis and hypertensive heart disease. The other, age 46, had been suffering from malignant hypertension at the time of her coronary attack.

"The conclusion seems warranted," the investigators comment, "that some factor associated with the menstrual cycle possibly protects young women against acute myocardial infarction."

CHOLESTEROL MEASUREMENT MOST PRACTICAL INDICATOR OF FAT METABOLISM DISORDERS

Measurement of serum cholesterol remains the most practical laboratory method of aiding in the identification of people with gross disorders of lipid metabolism which predispose to coronary disease, findings of an NHI grant-aided study conducted by investigators at the Harvard School of Public Health show.

The scientists, Dr. Eleanor Y. Lawry, Dr. George V. Mann, Ann Peterson, Alice Wysocki, Rita O'Connell, and Dr. Frederick J. Stare, compared cholesterol and lipoproteins in 1,968 "well" American men and women with those of 273 men who had suffered heart attacks, 141 men who had angina pectoris, and 23 women who had suffered heart attacks. This study differed from the Cooperative Study of Lipoproteins and Atherosclerosis, reported in 1956 and in which the Harvard group participated, in that the Cooperative Study was concerned with measurements in persons when they were well and in whom coronary disease developed during a subsequent period of observation.

Reporting in the American Journal of Medicine, the Harvard group presents data indicating that the association between

coronary disease and disordered fat metabolism can be most readily demonstrated with the serum cholesterol measure which, they explain, is as strongly related to coronary heart disease as the more technically complicated, expensive lipoprotein measurements.

Subjects studied were Americans, predominantly of the middle class, representing a variety of business and professional organizations, with emphasis on urban sedentary executives. Of the "well" people, 1,534 were men and 434 women. The "well" were those who showed no measurable evidence of coronary disease. Actually, nearly all adult Americans are believed to have some degree of coronary atherosclerosis.

"The most notable attribute of the serum lipid measurements of a large group of individuals is the great range of values observed among people of similar age, sex, and health status," the investigators report, stating that this variability appears to represent the manifestation of some regulatory mechanism that is poorly understood, and that the explanation of this variation may be the clue to an understanding of the causation of atherosclerosis.

Among the other findings from the Harvard study are the following:

The 273 men with heart attack history had higher levels of both cholesterol and lipoproteins than age-matched men without obvious disease.

In the 23 women with heart attack history the blood lipids were similar to those of age-matched men with heart attack histories. They were higher than those of age-matched women who had not suffered coronary attacks.

Between ages thirty and fifty the men had higher serum cholesterols than the women, but after fifty this situation was reversed, they found. An agewise increase which was seen in serum cholesterol was more pronounced in the women after age 30 than in the men.

The difference between the blood lipids of the "well" subjects who showed no overt evidence of coronary disease and those who did (angina or coronary attack history) was too variable and slight for measurements of these lipids to be of any value for predicting the development of coronary disease in individual subjects.

HIGH BLOOD PRESSURE

DIGITALIS-LIKE ACTIVITY IN BLOOD FOUND INCREASED IN PATIENTS WITH HYPERTENSION

A "system" of heart-stimulating substances normally present in all mammals has been found to be abnormally active in patients with essential hypertension.

An English physiologist observed four decades ago that when the beat of a frog heart isolated in a salt solution began to fail, adding mammalian blood plasma to the solution would restore the force of its contractions. This, and later experiments using mammalian hearts suggested the existence of digitalis-like substances in normal mammalian blood and tissues and prompted a search for them by Dr. Stephen Hajdu and co-workers of the National Heart Institute.

Because the isolated frog heart responds in a characteristic way to heart-active substances like digitalis, its use as a research tool was important in the sequence of events leading to the discovery of cardiotonic substances in mammals. Thus, Dr. Hajdu and his co-workers used an assay method of their own design, based on the response of the isolated frog heart to digitalis, to test tissue extracts for digitalis-like activity.

The first results of their work was the discovery of a specific fatty substance of a group called lysolecithins, which can be detected not only in blood, but also in beef adrenal glands, heart, and liver. The description and isolation of this cardiotonic material was published by Drs. Hajdu, Herbert Weiss and Elwood Titus in the Journal of Pharmacology and Experimental Therapeutics.

This material is responsible for only a small fraction of the total digitalis-like activity of serum; a large part is caused by a complex protein system. The protein system is present in all normal humans, but is increased in patients with hypertension. Drs. Hajdu, Edward Leonard, and Robert Akers reported this in Circulation Research.

Although the new systems bear no chemical resemblance to digitalis, they are similarly cardiotonic, increasing the contractile force of heart muscle. The activity of the system closely resembles that of digitalis when compared in a special test preparation of isolated living frog heart.

The cause of essential hypertension, which represents about 95% of the total hypertension problem, is unknown, and the role of the heart-active substances found operating in this disease also remains to be clarified by further research.

CHLOROTHIAZIDE FOUND TO HAVE VALUE AGAINST BOTH HYPERTENSION AND EDEMA

Chlorothiazide, a powerful diuretic recently introduced for the treatment of edema, has been shown also to possess blood-pressure-lowering properties valuable in the treatment of hypertension.

On the basis of independent studies in normal persons and hypertensive patients, two separate groups of scientists, one in Washington, D. C. and one in Boston, have found that chlorothiazide not only lowers blood pressure by a direct action when used alone, but also greatly enhances the effectiveness of other anti-hypertensive measures when used in combination with them.

Both research groups have reported that the drug is effective by mouth, free of serious side effects, and selective for hypertension, i.e., it does not lower blood pressure in normal persons.

Dr. Edward Freis (an NHI grantee) and co-workers of the Georgetown University Medical School and Mount Alto Veterans Hospital reported the hypotensive effects of chlorothiazide in The Medical Annals of the District of Columbia. Dr. Freis' group subsequently reported that, when added to other measures commonly used, chlorothiazide caused an additional sustained reduction in blood pressure which averaged 13% in two groups of patients with hypertension. It also allowed the dosage of the other drugs, some of which cause unpleasant side-effects, to be reduced. Maintenance doses of chlorothiazide itself produced no significant side-effects and no evidence of developing tolerance, the group found. Dr. Freis feels that the greatest usefulness of chlorothiazide against hypertension lies in its ability to "sensitize" the patient to the effects of other antihypertensive measures.

Drs. William Hollander (an NHI research fellow) and Robert Wilkins (an NHI grantee) of the Boston University School of Medicine reported in The Boston Medical Quarterly that chlorothiazide often is particularly effective in cases of hypertension which have resisted other forms of treatment and, because of its diuretic action, in arterial hypertension complicated by a failing heart and edema.

The drugs and surgery previously used have not been "selective" for hypertension as chlorothiazide appears to be, the Boston scientists point out. They feel that this, its ability to potentiate the action of other hypotensive measures, and its apparent dual mode of action as a diuretic and a direct hypotensive suggest it is a uniquely useful and entirely new kind of drug for the treatment of hypertension.

ANGIOTONIN, NATURAL BLOOD PRESSURE RAISING SUBSTANCE, NOW SYNTHESIZED

Cleveland scientists have synthesized a blood-pressure-raising (vasopressor) substance apparently identical with a natural vasopressor present in the blood of persons with hypertension resulting from kidney malfunction. This accomplishment, reported in the journal Science, was made in NHI grant-supported studies by Drs. F. Merlin Bumpus, Hans Schwarz, and Irvine H. Page of the Cleveland Clinic Foundation.

The natural vasopressor, which is called angiotonin, was discovered in 1939 by Drs. Page and O. M. Helmer and, simultaneously, by a group in Buenos Aires. It was found to result from the action of a kidney enzyme called renin on a substance made by the liver. Subsequent research has shown angiotonin to exist in two forms, one active and one inactive in raising blood pressure.

The new synthetic angiotonin appears to be identical with the active form.

Dr. Page, the Cleveland Clinic Foundation's research director, explains that by making possible the preparation of large quantities of angiotonin, the new advance permits intensive study of the mechanism of arterial hypertension by relating the chemical structure of the vasopressor substance to its effects on the body's mechanisms of blood pressure regulation. Such studies could lead to the synthesis of "competitive blocking agents"--drugs which reduce high blood pressure of kidney origin by interfering with the vasopressor action of natural angiotonin.

BLOOD PRESSURE IN INFANTS ACCURATELY MEASURED BY FLUSH METHOD

Evaluation studies conducted by California investigators in 317 infants and children show the flush method for determining infant blood pressures to be a practical and relatively accurate

procedure in the office or clinic. The investigators, Drs. A. J. Moss, W. Liebling, W. O. Austin, and F. H. Adams, of the University of California School of Medicine in Los Angeles and the Harbor General Hospital in Torrance, California, also found that the flush method reveals the mean, or average, of the systolic and diastolic blood pressures. The study was conducted while Dr. Austin was an NHI trainee.

The flush method--first described by a German physician in 1899--has occasionally been suggested as a way of determining blood pressures in infants, whose small arm and leg arteries often do not transmit the "cue" pulse sounds through the stethoscope. Although some investigators have felt that the flush method is acceptable, many others have felt that it is impractical or inaccurate, or both. Conflicting opinion over whether the cue skin flush reflects the pressure during the contracted (systolic) or the dilated (diastolic) phase of the heart cycle has also impeded its acceptance by physicians.

As used by the California group, the flush method involves firmly wrapping the child's hand or foot with elastic bandage and then applying an ordinary inflatable cuff with attached pressure gauge (sphygmomanometer) near the upper margin of the bandage. When the cuff is inflated and the pressure bandage removed, the limb is comparatively pale--blanched of its blood by the pressure of the bandage. As the pressure in the inflated cuff is gradually allowed to fall, a blush of red will abruptly suffuse the blanched limb when the blood pressure in its arteries counterbalances the constricting pressure of the cuff. The pressure at the flush is read from the sphygmomanometer, and is the mean blood pressure of the subject, the studies have shown.

The California group, which has reported its findings in the journal Pediatrics, demonstrated the accuracy of the method by having paired observers record blood pressures simultaneously in the same subject--in both arms in one group of fifty infants and in both legs in another group of fifty. They also correlated flush pressures with accurate direct pressure recordings which they obtained in catheterizing the aorta and chambers of the heart. The statistical values obtained from these studies indicated a high degree of reliability, the scientists report.

SURGERY SEEN DESIRABLE FOR CERTAIN PATIENTS WITH SEVERE HYPERTENSION

Despite the introduction of new, highly effective blood pressure lowering drugs, many sufferers from severe hypertension may still derive greater benefit from sympathectomy, an operation which interrupts artery-constricting impulses from spinal nerve centers. This conclusion was reached by Drs. George B. Hutchison (an NHI trainee) and James A. Evans of Boston in a review of the experience of the Lahey Clinic with sympathectomy operations between 1950 and 1954, published in Surgical Clinics of North America.

The number of sympathectomies performed at the Lahey Clinic has fallen off sharply in the past few years (from 45 in 1950 to 8 in 1954) because, the Lahey physicians explain, the advent of effective drug therapy has increasingly tended to limit the need for sympathectomy to cases with the more severe forms of hypertension.

"In the majority of these (severe) cases the disease can be controlled as well with the hypotensive drugs as by sympathectomy," they state. "This, however, necessitates continued use of drugs, probably for the rest of their lives, continued periodic check-ups and repeated dosage adjustments. We have, then, three indications for surgical therapy (sympathectomy) among these patients: (1) failure to respond to drugs (a small group); (2) young persons in whom many years of tedious drug control would be less justified than the operative morbidity, and (3) patients who live in remote areas and can neither obtain reliable medical management locally nor easily return for follow-up on re-evaluation for operations."

Patients who evidence milder forms of hypertension may also be eligible for sympathectomy, these doctors feel, if they show evidence of failing hearts, arteriosclerosis impairing their heart or brain, or disabling headache, dizziness or fatigue that seems related to their hypertension.

DRUG BOTH RAISES AND LOWERS BLOOD PRESSURE IN HUMANS

Tetrahydrozoline, a synthetic compound of medical interest because it was found to constrict blood vessels and raise blood pressure in animals, has now been found to dilate blood vessels and lower blood pressure in humans.

In 1955 Dr. D. E. Hutcheon and his colleagues at Pfizer research laboratories presented the results of studies in several species

of laboratory animals showing that the effects of tetrahydrozoline resemble those of epinephrine (adrenalin), a related substance found naturally in the body. Both compounds similarly constricted blood vessels, raised blood pressure, and increased blood sugar. However, the actions of the tetrahydrozoline were more sustained, and apparently safer in their effect on the heart, the Hutcheon group found. For this reason they suggested that more research should be carried out on the effects of this drug "so that its possible use in the treatment of certain hypotensive states can be ascertained."

Drs. Frank Finnerty (an NHI grantee), Joachim Bucholz, and Robert Guillaudeu of Georgetown University School of Medicine and the District of Columbia General Hospital, now report from studies in humans that tetrahydrozoline has both excitatory and inhibitory properties. Presenting their findings in the Proceedings of the Society for Experimental Biology and Medicine, they state that "The high initial concentration of the drug causes vasoconstriction; when this excitatory action has worn off, a sufficient concentration of tetrahydrozoline to cause vasodilation may remain."

The investigators obtained the evidence on which they base this concept of tetrahydrozoline action in studies of blood pressure responses to the drug in 42 hypertensives, who received the drug in intravenous injections. They witnessed an immediate rise in blood pressure which reached its peak in 3 minutes. By 25 minutes the blood pressure had returned to control levels and continued then to drop below pre-injection level, where it remained for 3 hours.

When the drug was given by mouth to 34 hypertensive patients, the investigators noted no increase in blood pressure at all--only the decrease, which was seen in all but seven of the 34. "Lack of a pressor response following oral administration can best be explained by lack of complete absorption resulting in low blood concentration," they explain. "It is hoped that these studies will stimulate investigation of related drugs for their antihypertensive properties."

HEART FAILURE

RESISTANT EDEMA YIELDS TO EFFECTS OF CHLOROTHIAZIDE ON KIDNEY SALT TRANSPORT

Chlorothiazide, a new oral diuretic, appears from research at Columbia University and Presbyterial Hospital in New York to increase kidney excretion of water-retaining sodium and chloride in a manner different from other diuretics.

Drs. John Laragh and Felix Demartini report, in a summary of their NHI grant-supported studies which appeared in the journal Circulation, that small oral doses of chlorothiazide are effective in treating the edema (accumulation in the tissues of sodium chloride and water) of nephrosis and cirrhosis, as well as that of heart failure. They found it free of dangerous side effects, "additive" to the effects of other diuretics, and effective in patients who were resistant to the conventional mercurial diuretics.

Five of six patients with nephrosis and six of eight with cirrhosis were reported relieved of fluid accumulations in body cavities and tissues by chlorothiazide, after mercurial diuretics and other measures had failed. Fourteen of fifteen patients in congestive heart failure, four of whom had become resistant to mercurial diuretics, were reported similarly helped by chlorothiazide.

The findings that its effects were "additive" to those of the mercurial diuretics, that it works on patients resistant to mercurial diuretics, and other peculiarities of chlorothiazide suggest to the investigators the existence in the kidney of at least two separate sodium and chloride conserving ("reabsorbing") mechanisms on which chlorothiazide and the mercurial diuretics may act.

Drs. Laragh and Demartini believe that the unique effects of chlorothiazide on sodium and chloride transport mechanisms allow a more rational approach to the derangements of salt and water balance that occur in certain phases of most heart diseases.

PITUITARY DOES NOT GOVERN SALT-HOLDING HORMONE SEEN IN EDEMA OF HEART FAILURE

National Heart Institute studies show the pituitary gland, often called the body's "governing endocrine gland" because it regulates secretion into the blood of many of the body's hormones, to be unnecessary for the secretion of the sodium and water regulating hormone, aldosterone.

This finding, reported in Federation Proceedings by Drs. Wilmot Ball, Robert Bahn, M. Jay Goodkind, and James O. Davis of the Laboratory of Kidney and Electrolyte Metabolism may help narrow the search for the mechanism that regulates aldosterone secretion from the outer layer, or cortex, of the adrenal gland. An excess of aldosterone has been found to be involved in the edema that bloats the tissues of persons with heart, kidney and liver diseases. It is the belief of many scientists that medical understanding and control of the body's aldosterone-regulating mechanism would make possible more effective treatment of edema.

When Dr. Ball and co-workers removed the pituitary glands of dogs with circulatory disorders which had resulted in excess aldosterone and bloating edema, one of two things happened. Either the excess aldosterone in the dogs fell to normal and their edema disappeared, or the aldosterone fell--but stayed above normal--and the edema didn't disappear.

But when the circulatory defects were produced in dogs whose pituitaries had already been removed weeks before, their aldosterone secretion climbed, their kidneys retained sodium, and they swelled with edema fluids like dogs with normal pituitary glands.

Thus aldosterone was shown to be an exception to a general rule that the hormones of the adrenal cortex are governed by the pituitary gland. Although the body's "governing endocrine gland" may influence aldosterone somewhat, the mechanism that prompts its excess secretion in heart disease appears from these studies to lie elsewhere.

TOXICITY OF AMPHENONE LIMITS ITS USEFULNESS AS A DIURETIC

Scientists have been seeking a drug that counteracts the salt and water regulating adrenal hormone, aldosterone, because an excess of this steroid hormone is associated with the condition of excess body salt and water (edema) that afflicts millions of persons with heart, kidney, and liver disorders. A synthetic compound called amphenone has been suggested as possibly useful because amphenone has been found to suppress the production of steroid hormones by the adrenal gland in animals and in patients with aldosterone-producing adrenal tumors.

A group of Boston investigators has reported in The New England Journal of Medicine on the use of amphenone to treat intractable edema in three patients with cirrhosis, a liver disease in which edema fluid often accumulates in the abdominal cavity. The scientists are Drs. Stanley Wolfe (an NHI Research Fellow),

Bernhard Fast, James Stormont, and Charles Davidson, of Harvard Medical School and Boston City Hospital.

Amphenone, they found, did have the desired effect on the edema in this disease--the patients lost salt and water. But the disadvantages of unwanted side effects with this particular drug appeared to outweigh any advantages of its use in treating edema. The patients tended to become drowsy and confused, and occasionally developed tremors and a state resembling the early stages of hepatic coma, a condition of depressed responsiveness that sometimes occurs in severe liver disease.

"Side reactions from prolonged administration of amphenone severely limit it as a therapeutic agent in edema states," they report. "However, if less toxic analogs of equal biologic potency can be synthesized, the results of this study suggest their potential usefulness."

SURGERY

MAJOR ADVANCES MADE TOWARD SAFE DIRECT VISION SURGERY ON AORTIC VALVE

A major obstacle to the safe and effective repair of the heart's aortic valve has been overcome by a technique that supplies blood to the heart muscle during heart by-pass operations without using the normal coronary artery supply lines.

Because the two openings, or "ostia", through which the coronary arteries receive their blood are located right at the aortic valve--in the very bases of its leaflets--the danger of coronary damage has challenged past attempts to expose this valve. Exposing these openings to free air results in coronary air embolism, ventricular fibrillation, and death.

With use of the new technique of "retroperfusion of the coronary sinus", developed experimentally in NHI grant-supported animal studies by Dr. Gumersindo Blanco and co-workers of the University of Puerto Rico, the valve can be safely exposed. The need of the heart muscle for oxygen is satisfied by pumping blood "in reverse" into the coronary sinus, the vein that normally drains the heart, and also the danger of air being drawn into the ostia is averted.

The value of the technique has now been demonstrated in the successful repair of heart defects in human patients by a surgical team at the University of Minnesota Medical School. The investigators, Drs. V. L. Gott, J. L. Gonzalez, M. N. Zuhdi, R. L. Varco, and C. W. Lillehei, have reported their research (which was aided in part by NHI grants) in the journal Surgery, Gynecology, and Obstetrics. They state: "The method of back perfusion via the coronary sinus has been tested in seven clinical cases to date. In all instances the method appeared to protect the myocardium well against anoxia and coronary air embolism permitting direct vision reparative surgery for aortic regurgitation, ruptured sinus of Valsalva, aortic-pulmonary septal defect, and complete transposition of the great vessels. The human heart responded well to this altered environment for periods up to 15 minutes."

The Minnesota group is now testing an additional modification which they feel may extend the usefulness of retroperfusion still further. They are combining retroperfusion with potassium-induced asystole--a technique of deliberately stopping the heart developed by the British surgeon Dennis Melrose. With the Melrose method of induced asystole the heart is not only relieved of its pumping job, but completely stilled by an injection of potassium citrate into the coronary arteries. This further reduces the heart muscle's oxygen demands, and may further

extend the surgeon's working time. It also offers the safety advantage of working in a still operative field.

ROUTE INTO HEART VIA MOUTH USED 500 TIMES IN PATIENTS TO DIAGNOSE HEART DISEASES

National Heart Institute surgeons in 1954 introduced into the U. S. a technique for entering, by way of the windpipe, the hitherto almost inaccessible left chambers of the heart. Since then they have been engaged in the first extensive research and clinical application of this valuable diagnostic approach.

Called "transbronchial puncture," the approach has been described by Drs. Andrew G. Morrow, Eugene Braunwald, and Herbert Tanenbaum in the journal Circulation. Reporting subsequently at a meeting of the American College of Surgeons, these investigators told of the safe and successful use of the transbronchial puncture in more than 500 patients in the NHI Clinic of Surgery. They also disclosed some modifications of the method which will expand its usefulness in the diagnosis of heart disease.

With the transbronchial technique, a slender tubular instrument called a bronchoscope is introduced down the throat to the dividing point of the two main branches of the windpipe. A two-chambered needle connected by a hollow tube to a pressure recording device is inserted down the bronchoscope and a puncture made just to the left of this dividing point, below which lies the heart's left auricle. A flexible plastic catheter tube is then slid through the needle into either the left auricle or on through the mitral valve into the left ventricle. Through this catheter, pressures may be measured, dye injected, and blood samples drawn to provide data important in the diagnosis of defective valves or holes in the heart's dividing partitions.

The modifications allow removal of the steel bronchoscope and its needle after the puncture is made, leaving behind only the threadlike catheter emerging from the patient's heart and mouth to be attached, at the surgeon's convenience, to his recording, injecting and sampling devices.

Thus the patient can relax and move about in comfort between widely spaced diagnostic procedures, and the surgeon can measure the effects of drugs, exercise, and other procedures and agents on blood pressures inside the left heart over prolonged periods of time while only the tiny catheter remains available to him as a communication with the heart's left chambers.

For more than 25 years, physicians have been probing into the right side of the heart simply by threading the catheter through a needle in an arm vein and pushing it "downstream" with the flow of venous blood into the right heart. There is no such avenue available to the surgeon through blood vessels into the left heart, though--it receives all its blood from the lungs. Before the transbronchial puncture, the only access to the left chambers (short of actual surgery) was by the more hazardous method of puncturing the chest wall with a long needle.

A German physician in 1950 was probably the first person to successfully attempt transbronchial entry to the heart. The technique as applied at the NHI was developed in a collaborative effort between Professor P. R. Allison, a surgeon in Leeds, England, and Dr. Morrow, Chief of the NHI Clinic of Surgery.

Using the transbronchial approach to the left side, together with the older arm vein approach to the right, both sides of the heart can be simultaneously catheterized for interpretation of pressure gradients, currents, oxygen differences, and indicator dye dilutions across damaged heart valves and between any of the four chambers.

SUCCESSFUL DIRECT VISION OPERATION FOR MITRAL INSUFFICIENCY REPORTED

The mitral valve is the most susceptible of the heart's valves to damage from rheumatic fever. Scarring from this disease may injure the valve in either, or both of two ways. It may partially fuse the two mobile lips, restricting the flow of blood through them (mitral stenosis). Thousands of stenosed mitral valves have been repaired by separating the fused lips with an index finger (to which a special knife is sometimes attached), introduced from above through the left auricle.

Much less approachable is the condition wherein rheumatic fever has thickened and roughened the valve so that it cannot close completely (mitral insufficiency). Repair of this condition in a 32-year old woman by suturing the valve under direct vision was recently reported in the Journal of the American Medical Association by NHI grantees Drs. K. A. Merendino and R. A. Bruce of the University of Washington, Seattle.

There have been no previous cases of mitral insufficiency reported in the medical literature as repaired by open heart surgery, Drs. Merendino and Bruce state. "This case has proved beyond doubt," they conclude, "that mitral regurgitation is a

remediable lesion... With some surgical procedures, it is necessary to perform a large number of operations to test their validity. From this single experience alone, it is believed that acquired mitral regurgitation is now in the realm of correctable lesions."

MEASURING DILUTION OF DYE INJECTED INTO HEART SHOWS EXACT LOCATION OF DEFECTS

The most common category of congenital heart disease--openings in the heart's middle partitions that let blood leak from its left to its right chambers--can be exactly located by a technique of injecting an indicator dye into the left chambers.

This "dye dilution" method, developed by Drs. Eugene Braunwald, Herbert Tanenbaum, and Andrew Morrow of the National Heart Institute, is described in a symposium on diagnostic methods published in the journal Circulation.

The approach is unique because it places the dye injection on the left side of the heart at or near the leak and in the path of its outflow. Conventional techniques, involving the sampling of blood from right heart chambers, sometimes fail to provide sufficiently accurate pre-operative information, the scientists explain.

Injected into the left heart of a normal subject, this dye is pumped, along with freshly oxygenated blood from the lungs, through the great aortic artery which arches from the top of the heart to supply all body tissues. As the dye flushes through the tiny arteries of an earlobe, darkening it momentarily, its concentration and transit time are accurately measured by an "ear oximeter". This is a tiny photoelectric cell attached to one surface of the earlobe. It responds to variations in the strength of a beam of light shining through the lobe from the opposite side. This beam is momentarily clouded by the dye flushing through, and the response of the photoelectric cell is projected on the screen of a cathode-ray oscilloscope, from which it is photographed. The dye concentration may also be measured directly in arterial blood obtained through a needle.

Recorded from normal subjects, this line of dye concentration in the ear is seen as a smooth ascending and descending curve.

When the heart is leaking from left to right side, a double peak is seen on the curve, because some of the dye being injected into the heart is immediately shunted through the hole and sidetracked to the lungs, with a time delay on its trip to the earlobe.

The shape of the curve in relation to the location of injection in the left heart tells the surgeon exactly where the hole is--information of value to him in mapping his operation, since distances of fractions of an inch are often critical.

Three NHI laboratories contributed to the development of the method. Dr. Braunwald was with the Laboratory of Cardiovascular Physiology, Dr. Tanenbaum with General Medicine and Experimental Therapeutics, and Dr. Morrow with the Clinic of Surgery.

The scientists report using left heart dye injection for precise location of left to right shunts (deflection) in 85 patients at the Heart Institute.

They also reported finding the new technique of value in the diagnosis of heart valve disease because blood leaking back through improperly closing valves also deflected the curve of dye concentration.

In most congenital heart disorders involving a shunt, it is from left to right. This is because the pressure is normally higher in the left pumping chamber which drives fresh blood to all the tissues, than in the right, which pumps exhausted blood under lower pressure to the lungs. Defects which allow blood to leak from the left side to the right may cause the heart to overwork and ultimately fail. Well over 1,000 persons, most of them children, die every year in the United States from such causes.

TISSUE GROWING OVER FABRIC ARTERY GRAFTS RESISTS ATHEROSCLEROSIS

The sheath of connective tissue which grows over aortic artery grafts made of nylon or orlon fabric apparently stays free of atherosclerosis even when this disease thickens adjacent areas of the host artery, it has been found in NHI grant-supported studies at Baylor University.

The investigators, Surgeons Oscar Creech, George Jordan, and Michael DeBakey, have previously shown in both dogs and humans that grafts of artery segments from another subject (homografts) will develop atherosclerosis along with the host artery. In the new studies, as in the earlier ones, the surgeons removed sections of the aorta in a group of dogs and bridged the gaps with substitutes--in this case substitutes of taffeta-weave nylon and knitted orlon, rather than segments of stored canine artery. Then, as before, they put the dogs on a high cholesterol diet known to result in atherosclerosis.

Ten of sixteen dogs so treated developed orange-yellow plaques in their coronary and aortic arteries and in the entrances to the rib (intercostal) arteries. The severity of the disease

varied directly with the degree of high blood cholesterol which the investigators were able to maintain in the animals.

The ten dogs grew the usual glistening transparent connective tissue sheaths over the cloth substitutes, but this lining in all ten remained completely free of the deposits which accumulated in the adjacent connective tissue lining of the host arteries. In discussing their findings in the journal Surgery, Gynecology, and Obstetrics, the authors conclude tentatively that "the results of this study suggest that synthetic aortic substitutes made of nylon and orlon, unlike aortic homografts, do not develop atherosclerotic lesions, although the adjacent aorta may be involved."

DIGITALIS INJECTIONS PRIOR TO "ICE BATH" SURGERY NOW LESSEN HEART FAILURE RISK

The use by surgeons of hypothermia to reduce the demands of body tissues for oxygen during heart operations may be safer in the future with the application of new research findings from the National Heart Institute Clinic of Surgery.

Drs. Thomas A. Lombardo, Leo R. Radigan, and Andrew G. Morrow have found in animal experiments that the threat of heart muscle failure in such operations can be greatly reduced by injections of strophanthidin, a digitalis-like "heart tonic" from the seeds of the African Strophanthus shrub.

The use of hypothermia permits many otherwise impossible heart operations because, by lowering body temperature several degrees below normal (in an ice bath), it reduces the needs of most body tissues for blood-borne oxygen. This allows the surgeon a period of eight to ten minutes to clamp off blood flow through the heart's chambers and work inside.

Heart muscle failure sometimes develops, apparently because the heart muscle needs a more continuous oxygen supply than other body tissues. Even in the chill of hypothermia it soon weakens in the absence of blood and, when the surgeon restores flow through the heart, it may be flabby and fail to perform its pumping job effectively.

In their strophanthidin experiments the NHI investigators lowered the body temperatures of a number of dogs fifteen to twenty degrees below normal in ice baths. They then clamped off the blood flow into the dogs' hearts and opened their right ventricles, as if to perform surgery inside. Half of these dogs received three injections each of strophanthidin at five-minute intervals beginning fifteen minutes before circulation was interrupted. The other half did not receive the drug and served as controls.

Seventy percent of the dogs unprotected by strophanthidin died, either during surgery or within twenty-four hours afterward, showing flabby, dilated hearts and other evidences of heart muscle failure.

Less than eighteen percent of the strophanthidin-treated dogs died within twenty-four hours, and in three of these the researchers found evidence that heart muscle failure may not have been responsible.

"This rapidly acting digitalis preparation prevented the evidences of myocardial failure in most of the animals and thereby increased survival," the researchers explained. "The data indicate that short term digitalization enables the myocardium to withstand temporary anoxia (oxygen deficiency)."

These findings were published in the journal Circulation. Treatment with strophanthidin, or other digitalis preparations, has since become routine for patients undergoing heart surgery with hypothermia at NHI and many other medical institutions.

"LEAKY" MITRAL VALVES REPAIRED BY SUTURING THE MITRAL RING

Surgeons at the Presbyterian Hospital in Philadelphia have developed a procedure for correcting insufficiency, or "leakage" of mitral valves damaged by rheumatic fever. This method involves encircling and narrowing the ring which supports the leaflets of the mitral valve with a strand of cotton tape, thus making it possible for the diseased valve leaflets to extend across and cover the opening between the left heart chambers.

The investigators, Dr. Robert P. Glover, an NHI grantee, and Dr. Julio C. Davila, report in the journal Circulation their results in the first twelve consecutive patients treated by the method. Realizing that this lesion presents one of the most difficult problems to the cardiac surgeon, the investigators selected patients for these trials who were in the end stages of their disease. Nevertheless, the immediate clinical results were adjudged to range from fair to excellent in ten of the twelve. However, the advanced status of their disease had produced irreversible heart, kidney and liver damage which was fatal in five of the ten despite mechanical correction of their valve defects. The investigators feel that their procedure, or any other surgical procedure, would have little to offer such terminal patients.

In operations on thirty patients who were in less advanced stages of the disease, the investigators subsequently report a surgical mortality of less than 20% and very satisfactory long-term results. Two of the thirty are now living some three years

after their operation and about half of the remainder are one-half to two years postoperative. All of these patients have shown marked to remarkable improvement, the investigators report, and most have returned to useful occupations and essentially normal lives.

DOG HEART USED TO EVALUATE ELECTRIC SHOCK METHODS FOR RESTORING NORMAL HEARTBEAT

Ventricular fibrillation, the sudden failure of normal muscle coordination in the contraction of the heart's ventricles, is the most dreaded complication of cardiac surgery. Electric shock, applied through a pair of padded electrodes placed directly on the surface of the heart, is the accepted method used by surgeons to restore the lost rhythm, and has saved many lives when the "rough handling" of the heart often necessary in cardiac surgery has precipitated this crisis. Surgeons, however, have not reached any general agreement concerning the best voltage and duration of shock for restoring rhythm to the pumping chambers without seriously burning the heart.

For this reason, Drs. George Kaiser, Jerome Kay, and John Edgcomb of the National Institutes of Health undertook a study of the effects of the different voltages and durations of shock on the canine heart. Drs. Kaiser and Kay, now with the departments of surgery of Indiana University and UCLA Medical Schools, respectively, were with the National Heart Institute's Clinic of Surgery at the time of the study. Dr. Edgcomb is with the NHI Clinical Center Pathologic Anatomy Branch. Their report on the study appears in the Journal of Thoracic Surgery.

These investigators used ten-volt electric shocks to the heart to interrupt normal beat and produce fibrillation in the anesthetized dogs. They allowed the ineffectual ventricular tremors of fibrillation to continue for four minutes, and then massaged the hearts for two minutes. This routine produced a state of affairs in the dogs resembling a more or less typical crisis of ventricular fibrillation as it often occurs in human cardiac surgery.

In four groups of dogs, they used two voltages, 130 and 230, at two different durations, 1/4 second and 1/10 second to defibrillate the canine hearts.

They found all of the voltages used about equally effective in restoring normal heartbeat. However, the higher voltage of 230 had more of a tendency to burn the heart muscle.

"There were myocardial burns in all four groups," they reported. "However, there was an increased incidence of burns of the heart using 230 volts at 1/4 second compared to the other groups. The burns of the heart defibrillated with 130 volts were small and

not visible grossly. The only severe burns occurred in the heart defibrillated with 230 volts."

PLAN REPLACEMENT OF LEAKY AORTIC VALVE WITH FLAP OF WIRE AND NYLON

Replacement of the aortic valve of the heart with a substitute made of a circle of fine watch-spring wire covered with nylon is believed by Detroit surgeons to be feasible. In NHI grant-aided studies, an operation for implanting the watch-spring aortic valve has been tested in dogs, and new surgical tools and techniques for removal and replacement of the diseased human aortic valve have been designed.

Development and trial in dogs of a mobile nylon and spring-wire flap to supplement the work of leaky mitral valves was announced last year by Drs. J. H. Wible, L. F. Jacobson, P. Jordon, C. G. Johnston, and H. Hellems of Wayne State University and Detroit Receiving Hospital. More recently they reported successfully implanting the device to cover a leaky mitral valve in a 34-year-old housewife whose heart was failing.

The Detroit group now reports in the A.M.A. Archives of Surgery they have implanted a similar nylon-covered spring flap in the aortic valve position in a group of dogs. It has performed--opening and closing with each pulse of blood from the heart--with encouraging results for as long as twenty months in these animals.

The investigators feel that actual removal of the three cusps or leaflets of the diseased human valve and replacement with the watch-spring flap may be better than simply supplementing the leaky valve with the flap as they have done in their past experiments. They have designed an aortic punch to be used in impending operations to shear out through the valve leaflets a channel of standard size to be covered by the prosthesis.

OPERATION WHICH USES GOLF TEE-SHAPED PLUG FOR HEART LEAKS SAVES LIVES AT NHI

NHI research surgeons have devised a heart operation that prevents the heart failure and death that have previously resulted from rupture into the heart of a weak spot, or aneurysm, at the base of the aorta.

The new procedure, called "the golf tee operation" because a tee-shaped device of pressed plastic sponge is used to plug the rupture, was devised by Dr. Andrew G. Morrow, Chief of the Clinic of Surgery, and successfully performed by him on two patients at the National Heart Institute. The patients, doomed to progressive heart failure without the operation, were freed of all symptoms and are now leading fully active lives.

The rupture occurs through a weak spot in the innermost lining of one of three little pouches called the sinuses of Valsalva, located in the wall of the aorta where this great artery emerges from the top of the heart. The weak spot, which may be congenital, bulges out under the great pressure of blood in the aorta like a faulty inner tube before a blow-out. The blow-out sends blood under high aortic pressure surging down into the lower-pressure right auricle or the right ventricle. Heart failure and death usually follow within a short time.

The unique diagnostic facilities of the NHI's Clinic of Surgery established the location of a leak between the aorta and the right auricle in a 28-year-old soldier with a failing heart referred from Walter Reed Army Medical Center to NHI for further study. The basic experimental work in dogs which necessarily precedes adoption of any new heart operation was undertaken by Drs. R. Robinson Baker and Edward Sharp, while the patient was treated medically to forestall heart failure and await the development of the operation which was to restore his health.

The basic dog work is described in the journal Surgery, Gynecology and Obstetrics by Drs. Baker and Sharp, and by Dr. Eric Hanson, a visiting scientist from Stockholm, Sweden, who contributed throughout as an integral part of the research team. The golf tee operation as performed for the first time in the young soldier is described in the journal Circulation by Drs. Morrow, Baker, and Hanson, and by Dr. T. W. Mattingly of Walter Reed.

PLASTIC VALVE REPLACEMENT FOR HEART'S MITRAL VALVE SHOWS PROMISE IN ANIMALS

A plastic ball valve intended for use as a substitute for leaky human mitral valves has been developed and tested in dogs at the State University of New York College of Medicine. Its use in the human heart now awaits modifications to guarantee secure anchorage of the valve in the heart and to preclude the possibility of its generating dangerous blood clots.

The valve is a one-inch cylindrical shell of white teflon housing a freely-moving polished lucite ball. When sewed into the opening between the left auricle and left ventricle (the channel of blood flow normally regulated by the two mobile lips of the mitral valve) the housing replaces the excised mitral lips and the action of the ball takes over their function. When the ventricle dilates to fill, the ball drops down in its teflon housing, away from the mitral hole it plugs, and lets blood en route from the lungs flow in from the auricle above. Then when the ventricle contracts to pump this blood to the body, the ball is forced up to cover the mitral channel and prevent backflow.

The investigators who designed and tested the valve, Drs. M. C. Kernan, M. M. Newman, B. S Levowitz, J. H. Stuckey, and Clarence Dennis (an NHI grantee), report in the Journal of Thoracic Surgery that in the dog trials the valve has proven to be a "mechanically efficient, rugged replacement".

STIMULATING CARDIAC EFFECTS OF ADRENALIN FOUND REVERSED BY DIGITALIS IN HYPOTHERMIA

Instead of stimulating the heart as it ordinarily does, the blood pressure-raising drug, adrenalin, has been found to depress the heart when used together with the heart tonic, digitalis, in subjects under hypothermia.

Surgeon Theodore Cooper of the National Heart Institute's Clinic of Surgery, and biochemist Marion Cotton of its Laboratory of Chemical Pharmacology have found that the cardiac stimulant effects of adrenalin and four of its close chemical relatives (noradrenalin, isoproterenol, ephedrine, and phenylephrine) were either blocked or reversed by two digitalis compounds (digitalis and strophanthidin) in dogs cooled 15 degrees below normal. At normal body temperatures the cardiac stimulant effects of the adrenalin-like drugs were not affected by the digitalis, but as digitalis-treated animals were cooled, the effects of the adrenalin-like drugs were blocked, and at 86 degrees they usually caused a measurable decrease in the force of heart muscle contractions.

Treating heart patients with the digitalis heart tonics before cooling them for surgery was shown by earlier published NHI research findings to prevent the heart failure that was a major threat in heart operations using hypothermia. Thus "digitalizing" the heart surgery candidate before operation has become a common safety practice in some clinics, as in the Heart Institute Clinic of Surgery. The adrenalin-like drugs, because they constrict arteries and raise blood pressure, are also commonly used in low blood pressure emergencies such as shock that sometimes develop during surgery.

Thus the new findings, Dr. Cooper explains, have revealed a potential but avoidable hazard for the patient undergoing cardiac surgery under hypothermia.

Preliminary studies with methoxamine, a blood pressure-raising drug different in chemical structure from the five others studied, indicate that it is not blocked by the digitalis compounds. More research is needed to determine the performance of various drugs and combinations of drugs under conditions of hypothermia.

Drs. Cooper and Cotton reported their findings at a meeting of the American Society for Pharmacology and Experimental Therapeutics.

VENTRICULAR ANEURYSMS SUCCESSFULLY REMOVED BY SURGERY

The first successful repair of a ventricular aneurysm, the ballooning distention of a weak spot in one of the heart's pumping chambers, was reported by surgeons at the Hahnemann Medical College in 1955. A total of eight patients have now had this condition repaired by the Philadelphia surgeons. Seven have survived.

Reporting their experience with the operation in the journal Surgery, Gynecology, and Obstetrics, the surgeons, Drs. C. P. Bailey and R. A. Gilman (an NHI trainee), state: "These encouraging results from surgical intervention in ventricular aneurysm in sharp contrast to the dismal prospects with conservative management strongly suggest a more aggressive approach to the treatment of this very common type of heart disease."

Ventricular aneurysms usually result from coronary atherosclerosis--they develop in the area of the heart which was scarred and weakened by being deprived of its coronary blood supply. More rarely, the aneurysms are due to an inborn weakness or to a chest wound or violent injury to the heart.

Patients with this condition may do relatively well--sometimes as well as other heart attack victims who do not get aneurysms, according to the surgeons. "Generally", they point out, however, "the clinical picture is characterized by the persistent pain of coronary insufficiency, by the development of intractable heart failure, and by a tendency toward embolic issue (the formation of drifting blood clots) and sudden death."

In their operation, the Philadelphia surgeons clamp off the ballooning aneurysmal sac, loosely applying a special needle-toothed fenestrated clamp well beyond the limits of the diseased area and into healthy heart tissue. The loosely applied clamp allows them to slit the chamber and flush it of all dislodgable blood clots that might otherwise wash into the general circulation later, with fatal consequences. They then tighten the clamp, amputate the sac, and sew the cut edges of the ventricle together.

RADIOACTIVE KRYPTON GAS CAN BE USED IN DETECTING CONGENITAL HEART DISEASE

Animal experiments with krypton^{85} in the National Heart Institute's Clinic of Surgery have shown that this radioactive gas can be used to detect, for surgical repair, abnormal openings in the

heart's middle partitions through which the blood leaks from its left to its right chambers.

Using dogs with holes in the dividing partitions of the heart, Dr. Richard Sanders introduced the radioactive krypton into the left, or arterial, side, and then sampled blood from the right, or venous side of the heart. Since the two sides are normally separate pumps, the presence of the abnormal communicating hole was reflected in the appearance of a high level of radioactivity in the samples from the right side. In normal dogs these samples showed comparatively little radioactivity because most of the krypton was lost to body tissues in the long normal circuit through the arterial system.

To introduce the krypton^{85}, Dr. Sanders had the dogs breathe it from an ordinary rubber anesthesia bag. The gas was picked up by the blood in the lungs and carried to the left heart along with its oxygen supply. To draw the samples from the right heart, Dr. Sanders threaded catheter tubing through a neck vein and thence into the right heart chambers.

The radioactivity was measured by drawing the blood from the catheter into a syringe along with a measured amount of air. The krypton passed from the blood into the air sample, which was then analyzed for radioactivity by holding it over the window of a Geiger-Mueller tube.

The advantages of the new radioactive krypton method over conventional ways of detecting the left-to-right shunts lie in its greater simplicity, speed, and accuracy. The older, more conventional method which measures blood oxygen in the chambers of the heart is much less sensitive, often failing altogether to reveal fairly serious leaks. Another method recently developed in the National Heart Institute is much more sensitive than the oxygen one and somewhat similar to the krypton method but uses nitrous oxide gas. This has proven valuable, but analyses of the nitrous oxide samples is more complicated than that of krypton and takes at least twenty minutes per sample.

The comparative attributes of oxygen, nitrous oxide, and radioactive gases were discussed in a recent NHI symposium on diagnostic methods which has been published in the journal Circulation.

NEW KNOWLEDGE AND METHODS

NHI INVESTIGATOR DISCOVERS ENERGY VEHICLE THAT POWERS HEART DURING FASTING STATE

A little known substance called unesterified fatty acid (UFA) that increases in the blood during fasting has been shown to be the fuel that powers the human heart, and probably all other organs except the brain, between meals when energy is not available directly from digested food.

By measuring the UFA content of blood entering and leaving the heart muscle and various other body tissues, Dr. Robert S. Gordon of the National Heart Institute's Laboratory of Cellular Physiology and Metabolism demonstrated that the amount of UFA extracted by the heart from its blood supply during fasting is approximately sufficient to satisfy its energy requirements for pumping the blood. Radioactive tracer studies at NHI confirm the role of UFA as a fuel. When radioactive UFA was injected into the blood of fasting subjects, it was exhaled from their lungs as radioactive carbon dioxide, the spent "ashes" of burned UFA.

Proving the importance of UFA to the heart, Dr. Gordon's studies indicate that UFA is also a major transport form, in the fasting state, for the energy that powers life processes in all body tissues except brain. The brain seems to require sugar as a fuel.

The studies show that, as the calories entering the blood from a digested meal are exhausted, UFA is increasingly mobilized into the blood from body fat (adipose tissue). Without the UFA energy transport mechanism, the calories stored in body fat presumably would not be available to the tissues, and starvation would result in a few hours without food. The recent findings concerning UFA are reported in the Journal of Clinical Investigation.

TOXICITY OF STREPTOKINASE IN HUMANS MUCH REDUCED BY PURIFICATION

Infusions of streptokinase, a substance of bacterial origin, have been shown to activate an enzyme, plasmin, which liquefies clotted blood. This has suggested its possible usefulness for dissolving blood clots in treatment of disease. However, infusions of streptokinase, even in very limited doses, have produced heart damage in rabbits and have brought on fever, low blood pressure, and nausea in humans. Thus there has been some question about the ability of the human system to tolerate a circulating clot-destroying substance without harmful effects.

Drs. A. J. Johnson, A. P. Fletcher, W. L. McCarty, and W. S. Tillett of New York University School of Medicine and Bellevue Hospital have reported a series of clinical trials that give a brighter picture of the potential usefulness of streptokinase. Using methods developed to prepare streptokinase 6 to 7 times purer than previously available preparations, they reduced the toxicity of the substance to man approximately 95 percent. Their experiments, supported in part by NHI grants, are reported in Proceedings of the Society for Experimental Biology and Medicine.

Sixty-one patients were given small amounts of streptokinase. Because of the limited dosage, only 15 of the patients responded with activated plasmin. These 15, however, produced a high level of the clot-destroying enzyme. Following the infusion, and for some hours afterwards, their blood showed spontaneous clot destruction in 45 minutes to 3 hours. Most important, these patients had the same degree and incidence of toxic reactions to streptokinase as the rest of the patients who had received the same infusion but had not responded to this limited dose with the activated plasmin. Of the 15 patients who did develop the clot-destroying enzyme, 13 had no more than a 1^{o} rise in temperature and a small lowering of blood pressure. These results indicate that the toxicity accompanying use of streptokinase is the result of contamination rather than the effect of the clot-destroying plasmin itself.

Since intravenous infusions of streptokinase would be used as specific treatment in selected patients with cardiovascular disease, the possibility of heart damage from the substance was investigated by an extensive series of electrocardiograms following streptokinase infusions. The experimenters found no indication of heart damage in humans attributable to the streptokinase and conclude that high levels of circulating clot-destroying enzyme can be tolerated by humans without harmful effects.

FINDINGS FROM BASIC RESEARCH IN CARDIOVASCULAR PHYSIOLOGY CHALLENGE CLASSICAL THEORIES

The tension developed by the fibers of the heart muscle at each beat--not the amount of blood it pumps--governs its demands on the coronary blood supply for oxygen, it has been found by Drs. S. J. Sarnoff, E. Braunwald, R. B. Case, W. N. Stainsby, G. H. Welch, and R. Macruz of the National Heart Institute's Laboratory of Cardiovascular Physiology.

These findings, which challenge classic concepts of heart action in health and disease, were made possible through the group's development of a better way to keep an animal's heart alive and performing normally.

Studies of a heart taken from an animal and kept living allow measurements impossible in the intact animal, especially of controlled changes in the mechanical factors which regulate heart performance. In the past such isolated hearts have always been nourished by blood, or a salt solution chemically resembling blood plasma, which is circulated through its blood vessels and chambers from an artificial reservoir, such as a glass beaker. The performance of such hearts tends to fail progressively, presumably because the nutritional adequacy of the continuously re-used blood deteriorates with time.

In the new isolated supported heart preparation of Dr. Sarnoff and co-workers, the blood reservoir is not a glass beaker but a healthy anesthetized dog. The blood supply for the isolated heart comes from the leg artery of the sleeping dog and drains back into a neck vein to be oxygenated by normal lungs--its wastes to be disposed of by normal kidneys and liver.

"Unlike previous isolated hearts," the investigators report, "this preparation exhibits performance characteristics (pressures, outputs, and efficiencies) which are not readily distinguishable from the in situ heart."

"As a result of experiments with this preparation," Dr. Sarnoff explains, "it has been ascertained that the heart muscle's requirement for oxygen per minute does not depend on the amount of work it does but on the amount of pressure, or total tension, which it develops with each beat. The amount of blood the heart puts out apparently does not, per se, influence its requirement for oxygen."

The new findings showing that tension, not fiber length alone, determines oxygen consumption, and have far-reaching implications at both the theoretical and applied levels.

STUDY SHOWS CULTURAL FACTORS NOT RESPONSIBLE FOR FEMALE LONGEVITY

The increasing advantage of women over men in life expectancy is due to the naturally superior resistance of the female to degenerative diseases, not to a less stressful way of life, studies at the Institute for Social Research in Social Science of the University of North Carolina indicate.

The Reverend Francis C. Madigan and Dr. Rupert B. Vance made this finding through study of mortality data from population groups that are different in sex but similar in their roles in life. A report on their NHI grant-supported study was published in the journal Social Forces.

Teaching orders of Catholic Brothers and Sisters were selected for the study because of the close similarity between the ways of life of the different sexes in these groups. Similarly exempt from the stresses of family and financial responsibility, military service, and excesses and dissipations, both male and female groups had the same kind of wholesome living conditions, religious beliefs and practices, professional responsibilities, and medical care. Thus any difference in life expectancy found between the men and women in the study groups could be attributed largely to biological, rather than cultural factors.

Comparing mortality statistics from 9,813 Brothers and 32,041 Sisters with male and female mortality statistics in the general population, the investigators found the same kind of growing female advantage in life expectancy in the religious orders that exist in the general population. "Sisters were the most favored population in every age group," they report. "Their advantage over Brothers has swelled from .1 years of life in the first decade (1900 to 1909) to 5.8 years in the present decade (1950 to 1954)."

These findings do not support the widely held theory that greater stresses in his way of life are wearing out the male earlier than the female, the investigators conclude. The data indicate that biological factors play a far more important part.

The fact that the female advantage is increasing in civilized countries with the passage of time the investigators attribute to the successful medical advance against infectious diseases and maternal mortality. The greater constitutional resistance of women to degenerative disorders such as heart disease benefits them increasingly as the infectious diseases and maternal hazards are brought under control, they explain.

FIND TRANQUILIZING DRUG DEPLETES BRAIN OF HORMONE GOVERNING ALARM MECHANISMS

Reserpine, a tranquilizer from the Rauwolfia plant widely used to treat excited mental states, has been found to deplete the brain of norepinephrine, a hormone believed to play a part in regulating the body's alarm mechanisms. This finding was published in Science by biochemists Parkhurst Shore, Jaqueline Olin, and Bernard Brodie of the National Heart Institute's Laboratory of Chemical Pharmacology.

Injections of reserpine in rabbits depleted their brain tissues of eighty percent or more of the stored norepinephrine within four hours, the investigators found.

This indicates, they point out, that future attempts to explain the effects of the Rauwolfia tranquilizers must consider these changes in brain norepinephrine. The findings also enhance the value of the Rauwolfia drugs as tools for research on the biochemistry of brain function, in which norepinephrine is involved. Scientific thinking on brain biochemistry has already been influenced by past studies in which Rauwolfia played an important part.

Recently another brain hormone--serotonin--was also found by Heart Institute scientists to be released by reserpine. The theory has been proposed that the effects of the Rauwolfia drugs are mediated by the release of the hormone, serotonin, in the brain, causing the sedative effects and lowered blood pressure seen after administration of the drugs.

The new findings on norepinephrine release at first seem paradoxical, for norepinephrine is believed to function in the sympathetic, or "alarm" nervous system, which should cause just the opposite effects to those seen after reserpine. Sympathetic impulses excite and increase blood pressure, heart rate and other activities that ready the body for flight or combat. Why, then, does a drug which calms the mentally disturbed cause the release of two brain hormones with functions that mutually antagonize each another?

One possibility, the scientists explain, is that the effects of norepinephrine, unlike those of serotonin, are not expressed when the drug releases it. If this is true, release might just deplete the working supply of norepinephrine and give a further advantage to serotonin's tranquilizing influence. Another possibility is that the norepinephrine release is just compensatory--a kind of "defense" by the sympathetic nervous system against the overshadowing activity caused by serotonin release.

These are just possibilities, the scientists point out. Continuing studies of the interplay of such substances as serotonin and norepinephrine--studies in which reserpine, one of the Rauwolfia alkaloids, is used as a tool--may supply facts which will make possible definite answers to the roles played in the brain by serotonin and norepinephrine.

ANTICOAGULANT FACTORS EXTRACTED FROM HUMAN BLOOD PLATELETS

Two anticoagulants which may be involved in human hemorrhagic diseases have been obtained from human blood platelets by Dr. Theodore Spaet, an NHI grantee at Montefiore Hospital in New York.

One of the new anti-clotting factors appears from his fractionation studies to be fatty in nature and the other to be protein, Dr. Spaet reports in the Journal of Applied Physiology. Both prevent clotting by interfering with the formation of thromboplastin, a forerunner in body tissues of fibrin, the essential cohesive part of blood clots.

In the past, much attention has been given to components of blood platelets that promote, rather than prevent, blood coagulation. Last year Dr. Spaet's group, and another group of scientists, reported in independent publications the finding that high concentrations of these tiny corpuscles have an anticoagulant effect.

"It is probable that the platelet anticoagulants have biological significance," Dr. Spaet reports. "Conditions characterized by massively increased blood platelets often display hemorrhagic manifestations resembling coagulation disorders. The concentration of platelets in the hemorrhagic thrombocythemias (diseases involving excess platelets) is well within the anticoagulant range. It is more difficult to evaluate the role of platelet anticoagulants in the normal hemostatic process. Perhaps they serve to moderate the coagulation process when local concentrations of platelets become excessive."

FIND REGIMEN OF STEROIDS WHICH RELIEVES MAJORITY OF NEPHROTIC PATIENTS

Research physicians at the National Heart Institute have described a regimen of therapy with prednisone or hydrocortisone thus far successful in the majority of nephrotic patients treated. Their findings, reported in the Journal of the American Medical Association, may help resolve current differences of medical opinion regarding use of the cortisone-like steroids against nephrosis.

The scientists, Drs. Howard Goodman and James Baxter, have tested their systematized mode of treatment in 22 nephrotic patients studied for from fifteen months to three and one-half years in the research clinical center of the National Institutes of Health.

Thirteen of the twenty patients fully recovered during or following steroid treatment. Their edema--the bloating of body tissues that characterizes nephrosis--subsided quickly, usually within a week or two, but this alone was not counted by the investigators as evidence of improvement. Two other more definitive symptoms--blood albumen deficiency and protein in the urine--also disappeared, usually within a month of oral therapy with either drug.

Four additional patients were greatly improved by the end of the study but some remaining kidney disease was evidenced by urinary protein and subnormal blood serum albumen.

The remaining 5 of the 22 were counted as unimproved, even though edema had been relieved in several, because there was no change in their serum albumen or urinary protein. Four of the five subsequently died.

Nephrosis is a common but poorly understood kidney disorder, most often seen in children, in which degeneration of certain kidney structures is accompanied by the appearance of protein in the urine and the development of edema. Drs. Goodman and Baxter point out that it is also common in adults, where the same combination of symptoms is often assumed to be due to nephritis.

Successful use of the cortisone-like steroids against nephrosis is not new--numerous reports of healing results with cortisone date back to 1950, but medical opinion of the value of such steroids varies widely. The steroids selected for the study--prednisone and hydrocortisone--are among those showing greatest promise against nephrosis. Hydrocortisone is an inflammation-suppressing hormone naturally present in the body and has been used in recent years to treat a host of inflammatory diseases. Prednisone is a newer synthetic chemical relative of the natural steroid hormones and has similar effects in the body.

Drs. Goodman and Baxter found the two steroids very similar in their effects when used in appropriate doses. Either drug was used for as long as necessary to achieve maximum clearing of protein in the urine--usually about a month. Then the dosage was reduced gradually. Both drugs had the unwanted side effects almost always associated with extensive use of the cortisone-like steroids.

Half of the patients in the study were children. Age, the investigators found, made no difference in the responses to the drugs, although in the past the outlook for adults with this type of kidney disease has been regarded as poor.

"Definite responses to steroid therapy occurred in children and adults--at least partial remissions occurred in approximately three-quarters of both groups," they report. "The high rate of remission in our group of adults suggests that steroid therapy is changing the outlook for these patients."

Concerning long-term outlook they caution against overoptimism. "Whether steroid therapy will change the long-term outlook remains to be seen, for half of the children might be expected to recover without it." In their study there were four cases

in which a good response to prednisone was later followed by complete relapse. Each of these recovered again after another course of prednisone treatment and remained well. None developed a tolerance for the steroid.

SURGICAL UNION OF RATS PROVIDES TOOL FOR AGING STUDIES

Scientists have long wondered whether pathological changes of age could be reversed by bathing the tissues of old animals in the blood of young ones.

To provide a tool for the study of this classical problem, investigators at Cornell University have attempted surgically to unite rats of widely different ages as parabiotics ("Siamese twins"), and then carry them in good health into old age. A report of their successful experiments, which were aided by an NHI grant, has appeared in the journal Gerontologia. The surgical procedure used involves joining anesthetized rats along incisions in their sides running from the base of the tail to the ear.

The scientists, C. M. McCay, F. Pope, W. Lunsford, G. Sperling, and P. Sambhavaphol, report maintaining united sibling pairs of rats, three to five months apart in age, for more than a year, with the older member of each pair reaching the last third of the normal rat life span. Rats 40 to 50 days old have also been united successfully by these investigators to rats between 470 to 480 days old. This is roughly equivalent to combining people 5 and 47 years of age.

The investigators found the tranquilizer, reserpine, valuable in preventing bullying of smaller and younger members of the pairs by the larger members--one of the most serious problems in this type of experiment.

FIND DEFICIENCY IN HUMANS THAT PREVENTS MANUFACTURE OF VITAMIN C IN THE BODY

A National Heart Institute scientist has discovered that there is a "missing step" in the body's ability to produce vitamin C, which may explain the cause of one of man's ancient diseases, scurvy.

According to Dr. J. J. Burns, a biochemist in the Institute's Laboratory of Chemical Pharmacology, the "missing step" is common only to man, monkeys, and guinea pigs. All other mammals have the ability to produce vitamin C.

Scientists have known for years that vitamin C plays an essential role in human nutrition. It keeps blood vessels, bones, and teeth in a healthy condition, and prevents and cures scurvy, a rare condition today but once a dread disease. Medical scientists, however, have been searching for years for the reason that man, monkeys, and guinea pigs, alone among the mammals, are unable to manufacture vitamin C for themselves, and must rely upon the food they eat for their supply.

A research finding which seems to explain this inability is offered by Dr. Burns in a report in the British journal Nature. He reports that man, monkeys, and guinea pigs are missing an enzymatic step found in all other mammals that converts a product of sugar metabolism in the body (L-gulonolactone) into vitamin C (ascorbic acid).

The missing enzyme system was uncovered by tracing similar amounts of L-gulonolactone administered to rats and guinea pigs. Previous work had established L-gulonolactone as a forerunner of vitamin C in the liver of the rat, an animal capable of manufacturing its own vitamin C.

It was observed in this experiment, however, that while the rats converted the forerunner, L-gulonolactone, into the vital vitamin C, the guinea pigs did not.

Further experimentation with human, rat, monkey, and guinea pig livers in the test tube corroborated these results.

Scurvy, which can be a fatal disease, is characterized by bleeding gums, anemia, and weakness. Though rarely seen today, scurvy presents an interesting history. Any record of its significant occurrence would have to span the years from the time of the Crusades to the First World War.

Scurvy has figured prominently in all wars. Whenever men could not obtain fresh fruits and vegetables (the chief source of vitamin C) for long periods of time, scurvy became a common and dreaded menace, often occurring in epidemic proportions. This was particularly evident among seamen during ocean voyages of exploration. Columbus' crew, it is said, suffered from scurvy.

Few deaths are reported from scurvy today, but the diet of a large part of the world is inadequate and many people are suffering from "borderline" deficiencies, according to medical authorities.

EXCESS ACTIVE SEROTONIN IN BRAIN CAUSES EXCITED STATE RESEMBLING LSD "PSYCHOSIS"

Animal experiments in the National Heart Institute's Laboratory of Chemical Pharmacology have raised the possibility that an excess of active serotonin in the brain, where this hormone may function as a conductor of nerve impulses, can cause marked effects on the brain.

By producing a sustained excess of the free and active form of brain serotonin, biochemists Parkhurst Shore and Bernard Brodie induced excited states in rabbits which could not be distinguished from the effects of LSD, a drug which in man causes temporary mental and emotional disturbances. A technical account of the rabbit experiments has appeared in the Proceedings of the Society for Experimental Biology and Medicine.

Evidence from earlier NHI studies indicates that brain serotonin normally exists largely in a stored and inactive state, and that it is released in minute quantities to act as an impulse conductor at the points of contact between nerve cells.

The LSD-like effects of excess free serotonin suggest to the scientists that this hormone might be producing central disturbances either by its excessive release at the points of nerve contact, or through a deficiency of the serotonin-destroying enzyme, monamine oxidase, that normally prevents its accumulation.

The validity of these speculations and the real meaning of the finding that excess serotonin produces emotional disturbances remains to be proven by continuing research on serotonin.

TOXEMIA, ECLAMPSIA AND ABRUPTION OF PREGNANCY ATTRIBUTED TO DAMAGED PLACENTAL TISSUE

The causes of toxemia of pregnancy remain uncertain, although its dreaded consequences--eclampsia and abruption (separation) of the placenta (afterbirth)--have been recognized as important causes of death in child bearing for centuries. Toxemia is usually signalled by an increase in blood pressure, swollen limbs and the appearance of protein in the urine. Although less dangerous if properly treated, untreated toxemia often leads to convulsions and coma--eclampsia. Abruption of the placenta may be preceded by similar warnings but often begins so suddenly, with severe abdominal pain and hemorrhage, that prevention is rarely possible.

Summarizing the findings of many years study, in the American Journal of Obstetrics and Gynecology, Dr. R. A. Bartholomew

and his colleagues at Emory University report that the one factor that appears to underlie the development of toxemia is an abnormal degeneration or "infarction" of placental tissue. Dr. Bartholomew, whose current studies are receiving NHI grant support, has studied toxemia for 25 years.

Dr. Bartholomew's group has been impressed with the consistent finding of black or brown areas of degenerating or dead tissue of varying extent in the substance or on the maternal surface of the placenta through which the mother's blood flows to supply nourishment to the developing child.

The placental degeneration is apparently brought about by spasm of muscular sphincters present in one or more placental veins. The constrictions necessarily block the exit of fetal blood from the involved placental areas, causing distention of the capillaries within the finger-like projections (villi) of placental tissue. The resulting expansion narrows or obliterates the blood spaces between the villi, and thus diminishes or shuts out the supply of maternal blood and oxygen. Degeneration of placental tissue ensues, liberating thromboplastin, a substance which brings about clotting of blood beneath the placenta and formation of strands of fibrin in the maternal blood stream. It is therefore important to know whether damaging effects to the mother result from possible poisonous products of protein or nuclear decomposition of placental tissue and/or mechanical effects of blocking of capillary circulation in various organs by fibrin strands.

Inasmuch as toxemia is initiated with the greatest frequency during labor, the reporting group believes that the hormonal changes that probably initiate labor, also cause placental venous sphincter constrictions in some cases and thus initiate toxemia. Oxytocin, the uterus-contracting hormone most active during labor, the investigators feel, is the logical trigger substance.

NHI STUDY CONFIRMS THAT, WITH AGING, VITAMIN B12 LEVEL DECLINES IN BLOOD

A gradual decline with age in the levels of vitamin B12 in blood serum, reported in a few earlier studies of smaller groups of subjects, has now been confirmed and evaluated in 528 healthy human subjects, ages 12 to 94, by investigators in the Gerontology Branch of the National Heart Institute and the Johns Hopkins School of Hygiene and Public Health.

The study, conducted by Drs. George W. Gaffney, Andrew Horonick, Kunio Okuda, Paul Meier, Bacon F. Chow, and Nathan W. Shock, was carried out in Baltimore where the Gerontology Branch is located. Results were published in the Journal of Gerontology.

The study included persons from public and private old people's homes, a penal institution, and the Baltimore population at large, as well as physicians and staff members of the Gerontology Branch and Baltimore City Hospitals. Effect of race, sex, dietary habits, socio-economic and other factors were considered. The findings suggest that, unlike other vitamins, B12 serum levels vary with age but not to any large extent with diet or socio-economic factors.

The clinical significance of the findings is that B12 deficiency is more likely to be a problem in older persons, the scientists explain. "Regardless of what reasonable value is selected as a level below which a B12 deficiency is likely to exist, a higher percentage of older people have such serum B12 concentrations," they report.

SEEK NORMAL ROLE IN MAN OF CHEMICAL FOUND IN INSECTS AND INBORN MENTAL DISORDER

A brain-stimulating substance called ortho tyrosine, hitherto found only in insects, has been implicated as a possible factor in the mental disorder, phenylpyruvic oligophrenia, in experiments conducted by scientists in the National Heart Institute's Laboratory of Clinical Biochemistry.

NHI biochemists Chozo Mitoma, Sidney Udenfriend, Donald Bogdanski, and Herbert Posner, whose findings have been published in the Proceedings of the Society for Experimental Biology and Medicine, explain that patients with this hereditary disease lack a liver enzyme normally responsible for breaking down phenylalanine, an amino acid necessary for life.

Phenylalanine is consumed in large amounts by most persons because it is present in nearly all protein foods, but only a part of it is essential for life--this the body uses directly to build new tissues. The rest is converted, in normal persons, into another amino acid--para tyrosine--by the liver enzyme that phenylpyruvic oligophrenics lack. The formation of para tyrosine from phenylalanine constitutes the first step in removing excess phenylalanine from the body. For the person with phenylpyruvic oligophrenia this avenue of disposal is blocked off. As a consequence, surplus phenylalanine is piled up in the tissues and disposed of slowly along pathways of metabolism that are little used in the body chemistry of normal persons.

The mental defect probably develops, the NHI scientists feel, because some phenylalanine byproduct is accumulating along one of these metabolic "detours" and poisoning the brain.

The NHI group began their exploration of the pathways of phenylalanine metabolism through the clues obtained by examining its end products in the urine. In phenylpyruvic oligophrenics, most phenylalanine ends up as chemicals not found in the urine of normal persons. One of these is called ortho-hydroxyphenylacetic acid. Its presence in the patients suggested to the NHI group that, in lieu of the normal para tyrosine route of disposal blocked in the liver, phenylalanine was instead traveling an ortho route--phenylalanine to ortho-tyrosine to ortho-tyramine to ortho-hydroxyphenylacetic acid in the urine.

Ortho-tyrosine, it seemed, might be responsible for retarding mental development. Or, it might be the next chemical step in the ortho route--perhaps the ortho-tyrosine was going into the brain to be changed to ortho-tyramine--the poisonous substance.

To probe this possibility tentatively, they injected some ortho-tyrosine into rats to see if it would act on their nervous systems. Analyses of tissues indicated it passed quickly into the brain where it was readily converted to ortho-tyramine. With this, the rats became excited, behaving as though they had been given benzedrine. Some had convulsions.

This did not prove that these ortho-compounds really were responsible for the mental defect, but it strongly underscored the possibility, and attempts are being made to demonstrate the presence of ortho-tyrosine and ortho-tyramine in the tissues of patients with this disease.

The investigators do not foresee replacement therapy with the missing enzymes as a consequence of their research on this disease. However, they feel that another possibility--that ortho tyramine may exist in the tissues of normal persons--is promising in terms of basic, rather than clinical, scientific knowledge. Related chemicals (amines) that are found normally in the body generally have important roles in life processes, and ortho tyramine, if it exists,may also have some important role.

FREE PASSAGE OF PENTOTHAL FROM BLOOD INTO THE BRAIN OF CLINICAL IMPORTANCE

The speed of action of the valuable intravenous anesthetic, thiopental ("sodium pentothal"), has been explained through research by Drs. L. C. Mark, C. I. Campomanes, S. H. Ngai, and E. M. Papper of Columbia-Presbyterian Medical Center, and J. J. Burns, N. Trousof, and B. B. Brodie of the National Heart Institute's Laboratory of Chemical Pharmacology.

Experiments in dogs by these scientists showed that the blood-brain barrier, a protective envelope surrounding the central nervous system, does not bar thiopental from the brain as it does most other foreign substances that appear in the blood. Only seconds after injecting thiopental into a leg vein, they found it had reached equilibrium between brain tissue and blood.

"The data indicate that thiopental passes into all parts of the brain with extraordinary rapidity, apparently unhindered by the blood-brain barrier," the scientists report in the Journal of Pharmacology and Experimental Therapeutics.

"The absence of a blood-brain barrier to thiopental has important clinical implications. It explains the commonly observed phenomenon of early respiratory depression, even to the point of apnea (arrested breathing) with thiopental anesthesia. It may also account for the catastrophic respiratory depression following the incautious administration of thiopental to patients with impaired vascular homeostatic mechanisms, as in shock."

PROMISING "PRESSOR" DRUG POSSIBLY HAZARDOUS FOR SOME PATIENTS

Caution in the use of methoxamine for low blood pressure emergencies is indicated by the findings of Drs. Lewis C. Mills and John H. Moyer in NHI grant-aided research at Baylor University.

Methoxamine is one of a number of new synthetic drugs that constrict blood vessels and raise blood pressure ("pressor amines"), acting like the natural adrenal hormones, epinephrine and norepinephrine. The unusually long lasting action of methoxamine after a single injection has suggested that it might be a valuable drug in the treatment of low blood pressure emergencies such as shock.

However, since a decrease in kidney function is a common accompaniment of the hypotensive state, it was important to determine whether or not methoxamine had deleterious circulatory effects, particularly in the kidney. For this reason, Drs. Mills and Moyer conducted a study in seven human volunteers of the renal and blood pressure effects of methoxamine, and compared the results with those from studies of the natural hormone, norepinephrine, and another synthetic drug, metaraminol, both of which are clinically useful as pressor amines.

Reporting in The American Journal of the Medical Sciences, the scientists present the following findings, among others:

Comparison of the renal effects of methoxamine to those produced by norepinephrine and metaraminol indicates that there is considerably more constriction of renal arteries for a given blood pressure rise when methoxamine is given. There is a significant lowering of the filtration rate and renal blood flow with use of methoxamine.

The investigators conclude that "In the treatment of patients with hypotensive emergencies, particularly when there is evidence of depression of renal function, pressor amines which have less renal effects than methoxamine, such as Aramine, phenylephrine, or norepinephrine would be a more logical choice for initial therapy."

SEROTONIN RELEASE IN LUNGS MAY CONTRIBUTE TO ALLERGIC REACTIONS SUCH AS ASTHMA

Serotonin has been found, with histamine, in the lungs of laboratory animals, indicating that this muscle-contracting "hormone" may share with histamine responsibility for allergic respiratory disturbances such as asthma. The NHI biochemists, Drs. Herbert Weissbach, T. Philip Waalkes, and Sidney Udenfriend, of the Laboratory of Clinical Biochemistry and the Clinic of General Medicine and Experimental Therapeutics also report from studies in rabbits that serotonin is released into the blood along with histamine in experimentally produced allergic reactions (anaphylactic shock).

Serotonin, like histamine, has known bronchial-constricting powers when injected into blood, but this compound had not previously been demonstrated in lung tissue. In the past, histamine released in the tissue affected has been held responsible for nearly all allergic reactions. Now, the investigators explain in the journal Science, the discovery of serotonin in the lung, along with the enzymes that make and destroy it, suggests both histamine and serotonin should be considered in explaining the pulmonary aspects of allergic responses.

Although both serotonin and histamine were found in the animals studied, the lungs of some, such as the mouse, contained much serotonin and little histamine. Others--the guinea pig, for example--had little lung serotonin and much histamine. This may help to explain why antihistamines tend to relieve the anaphylactic reaction in the guinea pig but are of little help in relieving similar reactions in mice, the scientists point out.

The investigators are now concerned with learning what role, if any, serotonin may play in human allergic disturbances. Technical limitations of the analytical apparatus used to detect serotonin in tissues have prevented demonstrating it in the human lung. It is expected that this problem will soon be overcome.

HIGHLIGHTS OF

PROGRESS IN ALLERGY

AND INFECTIOUS DISEASES

1957

Items of Interest on Program Developments

and Research Studies Conducted and Supported

by the National Institute of Allergy and Infectious Diseases

The year 1957 saw the development of two new programs in the National Institute of Allergy and Infectious Diseases. One was a field research project to be conducted in the Panama Canal Zone, the other a graduate training grants program to increase the number of investigators in certain critical areas of microbiology.

The Canal Zone study, to be known as the Middle American Research Unit, was authorized for 3 years and will give particular attention to the arthropod-borne viruses and their relation to certain infections common to both tropical and temperate zones. This project will be carried out in cooperation with the Department of the Army and the Panama Canal Zone Government.

Under the new training grants program, 30 applications for grants totaling $769,431 were approved for payment. Twelve of these were in the area of allergy and immunology, and the others were in tropical medicine and parasitology, mycology, and rickettsiology.

Prepared, January 1958

UPPER RESPIRATORY ILLNESSES

These diseases, such as influenza, the pneumonias and "colds," are our leading cause of morbidity, even in periods when influenza is not epidemic. They impose an immense tribute upon the life, health and economy of the United States. The U. S. National Health Survey estimated, for example, that there were 63 million new cases of respiratory disease involving at least one day in bed during one 4-month period (July 1 - October 26, 1957). The aggregate was 190 million bed days of disability. An average of nearly 6 million people were disabled each day because of respiratory illness during the week October 13-19, 1957.

VOLUNTEERS CHALLENGED WITH ASIAN INFLUENZA IN VACCINE EVALUATION

This study was undertaken by Drs. Joseph A. Bell, Thomas G. Ward, Albert Z. Kapikian, Alexis Shelokov, Thomas E. Reichelderfer, and Robert J. Huebner to determine whether a vaccine prepared from the newly isolated Asian strain of influenza virus would protect against illness produced by this virus. With the exception of Dr. Ward, who is from Lobund Institute, University of Notre Dame, all of the foregoing are from NIAID's Laboratory of Infectious Diseases.

Volunteers at the Maryland State Board of Correction's Patuxent Institution, at Jessup, Maryland, were given either vaccine or a harmless and inactive salt solution injection (placebo) on July 24 and 26, 1957. Neither volunteers nor observers knew who had received which product. The volunteers were healthy males, 21 to 57 years of age. (One-half were 21-25 years old.)

Two commercially prepared influenza vaccines were used, each containing killed virus particles of the Asian strain obtained from an outbreak in Japan in 1957. There appeared to be no essential difference between the two vaccine products in the degree of protection.

The infective agent which was sprayed into the nostrils of the volunteers, consisted of nasal and throat secretions from three of a group of Boy Scouts who became ill with Asian influenza during the 1957 Jamboree at Valley Forge, Pennsylvania. Laboratory studies indicated that no disease-producing agents other than Asian influenza virus were present in these specimens.

Antibody studies were made with blood serums collected before vaccination and before challenge with live influenza virus. None of the 23 placebo volunteers developed antibodies before challenge, whereas 22 (69 percent) of the 32 vaccinated volunteers did. Among the latter, 36 percent developed influenza following virus challenge. This compares with 78 percent of the placebo volunteers who became ill and 60 percent of the vaccinated volunteers who failed to develop antibodies following vaccination.

The investigators reported that 18 (78 percent) of the 23 placebo-inoculated volunteers contracted typical influenza after virus material was sprayed into their nostrils. This compares with 14 (44 percent) of the 32 vaccinated volunteers who became ill following virus challenge.

Dr. Bell cautioned against extrapolating these figures, involving a small number of subjects, to a situation in which large numbers are vaccinated and then followed for the occurrence of respiratory disease.

Both challenge groups were under constant medical supervision and were followed for at least 9 days after challenge. Medication consisted of aspirin and phenobarbital as needed; tetracycline was given only to three cases with pulmonary findings.

A typical case had a rather sudden onset about 38 hours after challenge. Initial symptoms were commonly a cold in the head, sneezing, severe headache, and fever. These and other manifestations, namely marked malaise, loss of appetite, scratchy throat, dry cough, ocular pain, marked muscle, bone and joint aches, and chills or chilliness, were most intense during the first day or so of illness and then gradually subsided. The illness lasted one day to six days with fever ranging from 100-103°F. The typical case was disabled, with the patient insisting on bed rest. A few patients began to feel better on the second or third day and then had a recurrence of marked clinical manifestations including fever. Hoarseness was observed in only one patient, nausea seldom occurred and none had vomiting or diarrhea. The cough occasionally persisted for two or more weeks after the acute phase was over.

In the daily physical examinations a typical case had the appearance of lethargy and weakness, and his face was either pallid or flushed. During the acute phase, the patient often refused to leave his bed for examination. The pulse and respiratory rate did not rise commensurately with the fever. No cardiac or bacterial complications were observed.

The study showed that vaccinated subjects were "less apt to develop clinical symptoms but if they did the symptoms tended to be milder and were less likely to be disabling or of as long duration."

The isolation procedures used during the studies were effective; no Asian influenza occurred in other inmates or in the custodial forces at the Patuxent Institution.

VACCINE OR PLACEBO OFFERED 6600 COUNTY RESIDENTS IN INFLUENZA SURVEY

Six schools in the Montgomery County, Maryland, area are cooperating in tests of Asian strain influenza vaccine. A total of 6,600 individuals (from 1340 families) were invited to participate in this study, a phase of NIAID research on influenza.

Half of the volunteers are receiving the Asian influenza inoculation, half an inactive placebo. NIAID has been conducting investigations of influenza and acute respiratory disease in community family groups since 1951. Thousands of Montgomery County residents have participated. Past studies helped the Institute further to establish that a vaccine made from killed influenza virus can give substantial protection against the disease, but only if the vaccine contains a virus strain similar to that causing the epidemic and only if it is given at least two weeks before exposure to the disease.

The Institute last spring had planned to discontinue the influenza phase of the respiratory virus studies, and initiated other important fundamental research on viral diseases before the present threat of Asian influenza became apparent. Through grant support, however, NIAID was able to arrange for the Johns Hopkins School of Hygiene and Public Health and the Maryland State and Montgomery County Health Departments to take over the Institute observations on these volunteer families and continue the controlled study. The staff of the Hopkins group, under the direction of Drs. Philip Sartwell and Raymond Seltser, worked closely with the Institute learning the techniques and methods that have been employed, so that the investigations may be continued in the same manner after the transition period. While NIAID will continue to collaborate with this group, the influenza study has been placed entirely under the auspices of the Johns Hopkins and health department epidemiologists. The grantees have now established their headquarters in Silver Spring, near the County study area.

NEW LAB TECHNIQUE AIDS DIAGNOSTIC SCREENING FOR INFLUENZA RESEARCH

A new method for rapid laboratory diagnosis of influenza has helped to expedite studies of this virus disease at a time when epidemic influenza involved major areas of the Far East and other parts of the world. The test was developed by investigators John Vogel and Alexis Shelokov of NIAID's Laboratory of Infectious Diseases.

The new "adsorption-hemagglutination" (AH) technique has proved of immediate value as a diagnostic aid in the laboratory. It makes use of the previously known fact that certain viruses cause red blood corpuscles to clump together (hemagglutination).

The customary method of isolation of influenza viruses employs embryonated chicken eggs and, less commonly, monkey kidney tissue cultures, which are inoculated with specimens from throat washings or swabs. The egg and tissue culture fluids are subsequently tested for the presence of hemagglutinating viruses by mixing them with red blood corpuscles obtained from chickens or guinea pigs.

In the new method, however, the red blood cells are added directly to the tissue culture tube inoculated with the patients' specimens. The reaction is then observed under the microscope.

When the reaction is positive, the red blood cells are seen to clump in the fluid and also to adhere to the surface of the sheet of tissue culture cells (adsorption) in a characteristic, easily recognizable pattern. The precise identification of the influenza virus can also be accomplished, using these tissue culture tubes, through inhibition of the reaction by specific antiserum against influenza A, including the Asian strain. Since a specific antiserum will inhibit only the type of virus against which it was prepared, inhibition is, in fact, identification.

The new technique is relatively simple, appears to be somewhat more sensitive, and allows positive identification of the virus considerably sooner. Twenty-three specimens from a July 1957 outbreak of suspected Asian influenza in the U. S. were tested by this procedure and the conventional techniques. Of the 23, the same 8 specimens were shown to contain influenza virus of the current Asian variety, but the identification by the new AH test was accomplished well in advance of the earliest egg isolation results.

The investigators point out that the new method is also of probable importance to fundamental research on virus-cell relationships. It is now being applied with promising results to studies of other hemagglutinating viruses.

NURSERY AGE GROUPS PRIME TARGET FOR ADENOVIRUS INFECTIONS

As a result of continuing studies of adenovirus infections in general populations Drs. Joseph A. Bell, R. J. Huebner and Wallace P. Rowe of NIAID's Laboratory of Infectious Diseases stress the fact that these infections are most prevalent in children. They emphasize the need for studies of the application to childhood age groups of adenovirus vaccines developed in the past two years and shown to be of value in military recruits.

Pre-school age children are the prime target of adenoviruses and the grippe-like illnesses they cause, the scientists pointed out. They based their estimates on several surveys, including a study of respiratory illnesses in 18,000 persons living in Montgomery County, Maryland, and in northern Virginia. These surveys indicate that not only do youngsters in the age group under six experience five times as much "undifferentiated, acute respiratory illness characterized by fever" as do adults in the general population, but they also have a much higher percentage of adenovirus infections. Infants and children under six have 15 to 20 times as many adenovirus infections as adults. Adolescents also were infected with adenoviruses much more often than adults.

These estimates represent the rationale for suggesting that adenovirus vaccines may have value in preventing some of the many acute respiratory illnesses that occur every year in 20 million American children in the nursery age group, and in many of the 40 million additional school children who have an intermediate experience with the viruses.

NIAID scientists suggest that the total number of adenovirus illnesses in the general population represent "certainly many times more than the several hundred thousand illnesses which conceivably could occur in military recruits. Furthermore, since effective prophylactic vaccines are generally regarded as eminently desirable in the latter group, it does not seem unreasonable to anticipate that similar efforts will be aimed at the prevention of the unknown but certainly larger amounts of illnesses due to adenoviruses in the general population. Before this can be accomplished, much more information is required concerning the specific roles of adenoviruses in illnesses accompanied by fever in young children and infants."

Equally important, said the scientists, will be the performance in field trials of suitable vaccines in preventing adenovirus illnesses in those younger age groups where most of the experience with adenovirus infections apparently occurs.

In the brief 4 years since the adenoviruses were first described by the National Institutes of Health scientists, representatives of this large family of at least 18 immunologically distinct human agents have been reported from many parts of the world. Only some of these have been sufficiently studied to establish their importance in causing different respiratory or ocular illnesses.

Research workers of the Public Health Service, Army and Navy have adequately demonstrated that killed adenovirus vaccine will prevent many of the acute respiratory illnesses which so commonly occur in military recruits. The vaccine contained adenovirus types 3, 4 and 7, which are highly prevalent in military recruits. Adenovirus types 1, 2, 3 and 5 are most prevalent in infants and children. A vaccine against these virus types may well prove useful in young children.

TUBERCULOSIS

A recent survey by the Public Health Service and the National Tuberculosis Association showed that there are still about 250,000 persons with active tuberculosis in this country. In 550,000 additional people the disease is inactive; but they require constant supervision. Another 1,200,000 who once had active tuberculosis are considered cured.

CONTINUING SEARCH FOR CHEMICALS TO DETOXIFY TUBERCULOSIS DRUGS

One of the most effective drugs in the treatment of tuberculosis is isoniazid. NIAID scientists Benjamin Prescott, Gladys Kauffman and Walter D. James of the Laboratory of Infectious Diseases have continued their studies of chemicals that combine with isoniazid to make it less toxic, so that larger, more curative amounts may be given.

These investigators have now found a number of effective detoxifiers of isoniazid in mice. The organic solvent, glycerine; the antibiotic, cycloserine; certain vitamins; and several amino and alpha keto acids have now been demonstrated to detoxify isoniazid and, in some cases, streptomycin. Typical of the detoxifying effects observed: if the vitamin pyridoxal was used with isoniazid, mice were protected when given up to 20 mg. of the drug, although 6 mg. in water, alone, are lethal. Many of the compounds also mediated against the toxicity that builds up with repeated small doses. The application of these findings to detoxifying these drugs in the therapy of human tuberculosis is foreseen.

CYSTS IN RABBITS CLUE TO LUNG LESIONS FOLLOWING TUBERCULOSIS CHEMOTHERAPY

Cyst-like formations are observed frequently in the lungs of pulmonary tuberculosis patients following effective treatment by currently used antituberculosis drugs. Since the mechanisms responsible for the development of these "cysts" are not known and potential significance as a source of flare-up of tuberculosis has not been determined, NIAID grantees sought clues by observing similar lesions in rabbits. At the Trudeau Laboratory, Saranac Lake, N. Y., Dr. William Steenken, Jr. has found that the blister-like cavities develop 3 to 8 weeks after the start of effective chemotherapy in rabbits infected with bovine tuberculosis. The process seemed

to involve a check-valve type of obstruction produced in the bronchi due to inflammation and swelling during the healing. Softening of the lung lesion probably occurred in the early stages of treatment. The softened material may have been evacuated through the bronchi paving the way for the cyst-like formation observed as an aftermath of effective chemotherapy. The rounded, thin-walled, radiolucent cavitary cyst appeared suddenly in areas previously occupied by dense solid lesions then usually soon lost rounded contours, shrank in size, and became small calcified scars.

PATTERNS OF ABSORPTION OF LABELLED DRUGS SHOWN IN TUBERCULOUS TISSUES

NIAID grantee Dr. Robert H. Ebert at the University of Chicago labelled two powerful anti-tuberculosis drugs--PAS and isoniazid--with radioactive carbon 14 to observe how they are absorbed in the body and compare their modes of action. Isoniazid was retained in tubercular lesions in experimental animals, while PAS penetrated the lesions but was not retained. Infected tissues, such as spleen and lung, apparently stored available isoniazid in direct ratio to the extent of the infection, while distribution of PAS was not affected in this way. These facts and other evidence may aid in developing better chemotherapeutic methods against tuberculosis.

RADIOACTIVE TRACERS REVEAL TACTICS OF TUBERCULOSIS ORGANISM

At Harvard University, NIAID grantees Dr. Emanuel Suter and Dr. Manfred L. Karnovsky, labelled the tuberculosis organism with radioactive carbon 14. They report significant differences between the way the tubercle bacillus and most other microorganisms encounter the body's defenses. Phagocytes, such as white cells, which usually engulf and digest foreign particles invading the body, did not disintegrate the tubercle organism, unless it was first shattered by high frequency sound waves. The respiratory rate of the bacillus did not change after phagocytation as it does in other microorganisms. The findings help to explain why tuberculosis is a notably stubborn disease. If the precise attack strategy of the bacillus can be revealed, it may be possible to reinforce the body defenses at a strategic point.

PROTECTIVE FACTORS AGAINST TUBERCULOSIS FOUND IN HUMAN SERA

First evidence that two fractions of human sera may contain protective factors against tuberculous infection has been obtained in NIAID grant-supported research by Dr. Charlotte Marker Zitrin and associates at New York University-Bellevue Medical Center, New York City. Using newer methods for separating different components of human blood the investigators isolated protective antibodies which were previously elusive. Inhibition of growth in the test tube of a strain of tubercle bacilli, and increase of the survival time of infected mice demonstrated the protective

quality of the fractions. "It was most interesting to find . . . an increase in survival time with Cohn fractions II-III and IV of tuberculous serum. The suggestive evidence for a protective effect of fraction IV is particularly noteworthy because antibodies have not been demonstrated previously in this fraction. . . . there may be protective antibodies in pooled human sera due to past infection, followed by continued antibody production."

ALLERGY-IMMUNOLOGY

Allergies afflict an estimated one person in ten in this country and rank third in prevalence among the chronic diseases. One of the most striking characteristics of human allergy is the diversity of its causes. They include, for example, inhaled materials such as plant pollens and molds; ingested substances such as drugs and foods; injected materials such as antitoxins made from animal serum; contact substances ranging from metals and plastics to dyes and a wide variety of chemicals.

Needs in this area of medicine are pressing. Most medical schools still give inadequate teaching in allergy to medical students. And many physicians could benefit from specialized training in this field; still others should be encouraged to seek careers in allergy research.

Progress in this direction has been made in the past year through the expanded program of the National Institute of Allergy and Infectious Diseases and also by the strengthened efforts of the Allergy Foundation of America, a private nonprofit voluntary organization.

TWO ALLERGENS SHOWN IN RAGWEED POLLEN AND TWO ANTIBODIES IN RAGWEED-ALLERGIC SERA

Dr. Bram Rose, NIAID grantee, and his associates at McGill University Medical School in Montreal, demonstrated in ragweed pollen at least two distinct substances which are significant in allergy to ragweed. Furthermore, they characterized two different kinds of antibodies in patients treated for ragweed allergy.

In experiments which led to these findings, ragweed pollen extract was separated into 7 fractions by means of electrophoresis. Four of these fractions were active.

They discovered that one fraction of ragweed pollen extract, when injected, induced antibody formation both in non-allergic and allergic persons. Other kinds of antibodies to ragweed pollen, however, are formed naturally in allergic individuals as defense against ragweed pollen invasion.

The significance of these studies is twofold. The fractionation of ragweed pollen extracts into many components will lead to improved preparation of ragweed extract used for the treatment of hay fever. Not only will it result in a more specific product, but also it will provide a better standardization of extracts used for testing and treating allergic patients. The recognition of different kinds of antibodies in the allergic patient is an important contribution to the study of the nature of allergic diseases.

COMPARISON OF REPOSITORY AND MULTI-VISIT IMMUNIZATION IN RAGWEED POLLEN ALLERGY

Many hay fever sufferers are unable to manage the 14 to 40 preseasonal injections required under present-day schedules for protection against attacks. For this reason a study was conducted to determine whether repository immunizations (single site immunizations from which injected substances are released gradually) could duplicate the results of multiple injections. Dr. Mary Hewitt Loveless (NIAID grantee) of the Cornell University Medical College and New York Hospital carried out a long-term study of 117 ragweed-allergic patients.

The effectiveness of the two procedures was found to be comparable, but the repository technique offers a great saving in time without decreasing the practical advantages of therapy or increasing the risk of overdose reactions. No abscesses, cysts, or malignant growths resulted from the 1200 repositories in which adjuvants, substances which have been found to enhance the protective effects of immunization, were used during the past 9 years.

Three criteria were applied in comparing the effectiveness of the two methods of immunization: the customary clinical estimate obtained from the patient at the end of each pollinating season, his daily diary of hay fever hours, and a controlled type of conjunctival test which gauged the acquisition of blocking antibody by the tissues.

The patients included in this study were selected on the basis of sensitivity tests to ragweed pollen only, and those individuals with allergies to other pollens, house-dust, or animal danders were excluded from the tests.

The conjunctival test provided a more accurate method for measurement of blocking antibody, the investigator reported, than the patient's clinical reports and recorded hay fever hours. It was suggested that this test could be utilized by allergists who employ only the skin and scratch methods of classification.

LEPTOSPIRA "BATTLE TACTICS" SPOTLIGHTED BY USE OF FLUORESCENT ANTIBODIES

Dr. John E. Tobie of NIAID's Laboratory of Immunology is employing fluorescent dyed antibodies in a study of Leptospira icterohaemorrhagiae. Leptospirae cause an acute, systemic febrile infection of man, known as leptospirosis, which is usually acquired by contact with material contaminated by the urine of infected animals, particularly rats. The leptospira under study kills guinea pigs in about 5 days. Tissue is quick frozen, and slices of about 3 microns thickness are mounted on slides and dried. Fluorescent antibody specific for this microorganism is applied; it couples with the fluorescent dye in the process. The labeled antibody will adhere to its specific pathogen but can be removed from other organisms by rinsing. Under the fluorescent microscope the leptospira organisms glow. The "battle tactics" of these pathogens become more apparent. The technique will show, for example, precisely where they localize in the cells.

NIAID FLOCCULATION TEST EXTENDED TO DIAGNOSIS OF ARTHRITIS

In a collaborative project with NIAMD, immunologists John Bozicevich and Jules Freund of NIAID extended a diagnostic test developed and used by them for trichinosis to the diagnosis of rheumatoid arthritis.

This simple, rapid diagnostic method, known as the bentonite flocculation test, is considered as accurate as the best of current tests for rheumatoid arthritis, but with the added practical advantage of producing results in a few minutes rather than days, and requiring only simple, easily available materials and equipment. The simplicity of the new test will make it possible for the average medical technician or physician's assistant to perform 100 or more such tests per day.

The flocculation test employs a type of colloidal clay known as bentonite, which is mixed with normal human gamma globulin. A drop of blood serum from the person being tested is added to a drop of bentonite-gamma globulin mixture on a slide. A positive test is demonstrated by the bentonite particles clumping (flocculating) within a few minutes, which is detectable when viewed under a microscope.

Eight out of ten cases of rheumatoid arthritis were detected by means of this test and false positives were less than two out of a hundred cases.

In a paper presented at the Ninth International Congress on Rheumatic Diseases in Toronto, Ontario, Dr. Joseph J. Bunim of the National Institute of Arthritis and Metabolic Diseases reported the results of clinical tests using this diagnostic procedure.

PURIFIED IMMUNIZERS FOR PERSONS ALLERGIC TO WASP STING

Doctors Mary H. Loveless and William R. Rackler have developed an improved wasp-sting immunizing agent during NIAID grant-supported studies at Cornell University and New York Hospital.

Presently available commercial immunizing agents are made by grinding the whole bodies of insects. The extraneous fragments contain proteins that may sensitize the recipient and lead to undesirable and perhaps dangerous reactions.

In producing a purified material the grantees employ only the venom sacs. The risk of variation in potency among individual sacs is minimized by using from 10 to 100 sacs as the starting material for a given series of dilutions.

Six wasp venom sacs are generally sufficient to give immunity to the allergic individual. This amount can be administered in a series of injections during a single prolonged visit. The most convincing criterion of immunity employed during extensive tests was the ability of the volunteer with a history of sensitivity to undergo planned or accidental stinging without untoward effect following a course of immunization.

LOCALIZATION OF TETRACYCLINES REVEALED IN BONE

The localization of tetracyclines in new bone growth was revealed by fluorescent microscopy. This study was conducted by Dr. John F. Tobie of NIAID's Laboratory of Immunology in cooperation with Dr. Robert A. Milch and Dr. David P. Rall of the National Cancer Institute.

During the course of some experiments involving the administration of antibiotics of the tetracyclines series (tetracycline, chlortetracycline, and oxytetracycline), it was noted that these drugs were deposited in skeletal tissues. Localization of the antibiotics in the body was determined by the yellow-gold fluorescent light emitted by these substances when stimulated by ultra-violet light. A blue light is emitted by ordinary fluorescence in uninvolved bone tissue.

When these tetracycline drugs were administered to animals parenterally, diffuse distribution was observed for a period of about 12-24 hours, after which time the drugs persisted in both long and flat bones. Examination of sections of fresh, frozen undecalcified bone by means of the fluorescent microscope revealed that the drugs localized in newly proliferated bone but not in established bone.

When once deposited in the skeleton, the tetracyclines apparently persist for prolonged periods of time. In rabbits, bone fluorescence was evident six months after a single parenteral dose of tetracycline. This persistence of the drug in bone for extended periods of time could conceivably be a health hazard. This study suggests that the tetracycline drugs may provide effective histological indicators in man of newly proliferated bone tissue.

PARASITIC DISEASES

Parasitic diseases are generally also known as tropical diseases because they are most prevalent in these areas; but they represent an important public health problem in temperate zones, too. The impact upon world health and economy is incalculable. These diseases kill or disable millions each year. Hundreds of thousands of our people are at risk as they live, work or travel in tropical or semi-tropical areas where parasitic infections are a constant hazard. More is at stake than the personal well-being of those directly exposed. With the dependence of the United States on tropical areas for essential raw materials and the tremendous investments of capital in the tropics, efforts to improve health standards there represent a prudent and farsighted undertaking and one which affects our national security. The NIAID is a world center for tropical disease research.

MALARIA SUPPRESSIVE DRUGS BLENDED WITH TABLE SALT TAKEN ROUTINELY AT MEALS

The normal, everyday dietary routine of salting food has been employed as a convenient and efficient method for malaria suppression by Dr. G. Robert Coatney of NIAID's Laboratory of Tropical Diseases. The antimalarial drugs, pyrimethamine and chloroquine, blended with table salt, suppressed malarial attacks in prison volunteers bitten repeatedly by infected mosquitoes. Sixteen volunteers took part in the trials and nine men served as controls. The volunteers were inmates of the U. S. Penitentiary at Atlanta, Georgia. The World Health Organization sponsored this study.

One group of eight volunteers used pyrimethamine-in-salt as food seasoning. Each man was bitten by 10 infected mosquitoes on the 14th day after dietary medication started. All were bitten again 7 days later and for a third time 7 days after that. The drug-in-salt regimen was continued for 18 additional days. The controls who received no medicated salt developed malaria 13-15 days after being bitten, whereas the men given the salt-drug mixture did not show symptoms of malaria until 28-40 days after the medicated salt was withdrawn from use. Similar results were obtained in tests carried out with the other antimalarial drug, chloroquine.

The salt-drug mixtures used in these tests were prepared by blending the average normal daily quantity of "ad lib" table salt with the daily requirement of either of the antimalarial drugs. Tests showed that the potency of the drugs survives cooking.

In undeveloped areas of the world, where open housing does not lend itself to malaria control through insecticide spraying, the dietary method of suppressing malaria may provide a cheap and practical means of protecting populations.

LAB TESTS SHOW NUCLEOCIDIN EFFECTIVE AGAINST FORMS OF AFRICAN SLEEPING SICKNESS

The era of enormous epidemics of African sleeping sickness may be over (in Uganda an epidemic once killed 200,000 people); but the existence of this disease requires continuing vigilance over millions of people. And in Africa today, over 4 million miles of fertile land lie idle and uninhabitable--the dominion of the tsetse fly and of the blood parasite, the trypanosome, which the fly carries.

Recently the first reports have been made of antibiotics, such as Stylomycin (Puromycin), which are effective against several species of trypanosomes. Now, parasitologist Eleanor Tobie and associates of NIAID's Laboratory of Tropical Diseases demonstrate that a new antibiotic, Nucleocidin, is effective in mice and rats against representatives of three groups of trypanosomes: it is possibly the first antibiotic found highly active against T. congolense, a parasite of cattle; it is effective, also, against T. equinum, a species found in horses; and against T. gambiense, one of the species causing sleeping sickness in man. (Whether or not certain animal species may be transmitted to and cause diseases in humans has not been definitely established.)

The amount of drug (given in one to three daily doses of 0.5 milligrams per kilogram of body weight of the test animal), the number of infective parasites injected, and the time they were allowed to multiply were among factors affecting results. Another consideration was the reported toxicity of Nucleocidin, but the investigators feel the deaths of only three of 230 mice, and perhaps a higher relative number of rats, might be due to toxicity. Early treatment with a single dose of Nucleocidin resulted in cures of nearly all of the mice and rats infected with one or another of the three representative species of trypanosomes. However, when treatment was delayed until the third day of infection with T. gambiense, for example, none of the mice was cured with a single dose of the antibiotic; but 73 percent were cured with three doses. When the number of trypanosomes inoculated was increased from 10,000 to 1,000,000 and treatment was withheld until the third day, even three doses did not cure.

LARVAE OF SCIOMYZID FLY MAY PROVE TO BE ALLIES AGAINST SCHISTOSOMIASIS

A new approach to control of the snail-borne disease, schistosomiasis (a major affliction of humans in some areas of the world), is suggested by work of NIAID grantees C. O. Berg and associates at Cornell. They foresee employing species of the sciomyzid fly in snail eradication. These flies hatch larvae which feed on and kill snails. The larvae showed "real promise" against several major types of snails which harbor the schistosomiasis parasite. The adult fly is found in humid, marshy areas. Little is known about it, except that it is not identified as a pest of humans or livestock.

The principal investigator published the first suggestion (1953) that snail eating is not an exception but is a widespread habit among these larvae, after he watched them attack snails, kill them and consume most soft parts in a few hours.

The grantees report "a gradually crystallizing opinion" that the predatory tetanocerine species offers greater promise than any other sciomyzid larvae for the biological control of medically important snails.

Some of the grantee work was conducted at NIAID's Laboratory of Tropical Diseases, where important varieties of snails are available in aquariums. (They were used recently in developing effective chemical compounds to aid in snail eradication.)

The grantees now have demonstrated that 30 species of the sciomyzid fly kill and feed upon snails. Twenty-two species were run through their life cycles in the laboratory. Plans for the future include a field test, perhaps in Puerto Rico, to determine whether sciomyzid species can be introduced successfully to appreciably lessen the population density of Australorbis glabratus, the snail host of the schistosomiasis parasite in South America and the West Indies. A colony of these snails were among those provided by NIAID for preliminary studies.

NEW ISOLATIONS OF VIRUS AS JUNGLE YELLOW FEVER MOVES SLOWLY TOWARD U. S.

In recent years the U. S. Government-supported Gorgas Memorial Laboratory in Panama and the Pan American Sanitary Bureau have directed their yellow fever studies toward a special problem. A wave of jungle yellow fever has been moving slowly northward through Central America and Mexico toward the southern United States where the Aedes aegypti mosquito, urban vector of the disease, is prevalent.

When the wave of yellow fever reached Guatemala in 1956, the PASB urged broad cooperation with the Gorgas Laboratory and also arranged with this Laboratory to attempt virus isolations from mosquitoes captured in areas showing yellow fever activity. Uncertainty as to the number of species and possible sub-species of jungle mosquito vectors is one of the gaps in knowledge of this disease.

Gorgas scientists reported recently the isolation of yellow fever virus for the first time from three species of naturally infected mosquitoes--Haemagogus mesodentatus, Haemagogus equinus, and Sabethes chloropterus collected in Guatemala. Identification of yellow fever virus was based on cross-immunity and mouse protection tests. Some 9,893 mosquitoes were used for virus isolation tests. Yellow fever virus was recovered 14 times from pools of 4,021 specimens of H. mesodentatus, three times from 1,686 H. equinus, and four times from 3,141 S. chloropterus. While these species of mosquitoes are found principally in Central America, H. equinus is quite generally distributed and has been collected in the southern part of the United States.

FEVER THERAPY POSSIBLE WITH MALARIA PARASITES KEPT YEARS IN DEEP-FREEZE

Low temperature preservation of malaria parasites has proved a satisfactory method of maintaining readily available supplies of these organisms which cannot be cultured in test tubes. Experiments by Dr. Geoffrey M. Jeffery of the Columbia, S. C. facility, Laboratory of Tropical Diseases, NIAID, indicate that little viability of blood parasites is lost when they are quick-frozen and stored at about minus 70°C. Preservation for at least two years appears feasible. As a general practice, however, more frequent replenishment of frozen stocks seems desirable.

In several instances frozen blood containing the parasites--previously difficult for physicians to obtain on short notice--has been shipped (packed in solid CO_2) to various parts of the country for use in treatment of neurosyphilitics. In the only report thus far received concerning the viability of this blood, the recipient came down with the fever after the parasite had incubated for 12 days--not unusually long for the Plasmodium vivax organism. The specimen had been preserved for 240 days prior to shipment.

All four species of Plasmodium of man now have been successfully stored for extended periods. Among 18 transmissions of the parasites recently reported, eight of the strains had been preserved in excess of one year, and three in excess of two years, including one strain preserved for 997 days. A sporozoite sample comprising 20 salivary glands of Anopheles quadrimaculatus mosquitoes infected with P. vivax (Chesson strain) was infective after being frozen for 785 days.

TICKS PRESERVE, TRANSMIT LEPTOSPIRAE IN TESTS: MAY BE VECTORS IN NATURE

Dr. W. Burgdorfer and associates of NIAID's Rocky Mountain Laboratory in Hamilton, Montana, have demonstrated the ability of ticks to preserve and transmit Leptospira pomona, suggesting that certain species of ticks may serve as vectors of leptospirosis in nature. Leptospirosis in man is an acute, febrile, systemic infection usually acquired by contact with material contaminated by the urine of infected animals. It is not generally fatal, although in some types of leptospirosis a mortality rate of 30% or higher has been reported.

In tests just reported, the soft-shelled tick, Ornithodoros turicata, was found to preserve and transmit leptospirae after feeding upon the air-sac membrane of infected chicken eggs or upon infected hamsters.

The hard-shelled ticks, Dermacentor andersoni and Amblyomma maculatum, both common parasites of livestock, were infected by a new technique. Glass capillary tubes were filled with a suspension of a culture of L. Pomona and placed over the hypostome of the ticks. The ticks fed readily and after 4 or 6 hours the majority of them were found partially engorged. Fourteen days later they were allowed to complete engorgement on normal weanling guinea pigs. Several of these subsequently became infected, indicating that these ticks are capable of transmitting L. pomona.

Ticks have a wide variety of hosts, many of them known to become infected with leptospirae. The data obtained with O. turicata, D. andersoni, and A. maculatum suggest that ticks may play a part in the maintenance and distribution of leptospirosis in nature and attention should be given to this factor in planning for control.

DEFENSE AGAINST PARASITES POOR IN GERM-FREE ANIMAL WITHOUT "BATTLE-EXPERIENCE"

Most animals are unsuitable hosts for parasites not common to their particular species, and soon immobilize such parasites through specific body defenses. This is not true, however, of the germ-free animal, as studies by Dr. Walter Newton of NIAID's Laboratory of Tropical Diseases reveal. Following the inoculation of germ-free animals with certain worm (helminth) parasites, sterile larvae from cultures of Nippostrongylus muris from the rat and of Nematospiroides dubius from the mouse, as well as sterile eggs of the tapeworm, Hymenolepis nana from the mouse, were found to develop to fertile, egg-producing adults in germ-free guinea pigs. In contrast, these parasites developed only poorly or not at all in conventional guinea pigs. The diet of the germ-free animal, which is different from that of the conventional one, was shown not to be the determining factor in these results. When sterile larvae of Trichinella spiralis were fed to germ-free guinea pigs, they also developed to adults and produced infective, musculature-invading larvae.

The development of rat and mouse helminths in the germ-free guinea pig suggests that factors associated with host specificity or natural resistance to the establishment of an "abnormal" parasite in a given species are absent or of reduced activity in the germ-free animal of that species. Also, it is apparent that these helminths do not require the presence of live bacteria in the gut of the host for apparently normal development and maintenance. This is of particular interest with regard to the nutrition of the tapeworm which does not have a digestive tract, and which presumably absorbs food through its external surface.

TOXOPLASMOSIS

Toxoplasmosis, an obscure parasitic disease, which at one time or another infects a high percentage of people in the United States, as well as in widely distributed areas of the world, can be an inapparent, mild, severe or fatal infection.

The most common type of acute toxoplasmosis is a mild disease resembling glandular fever. Other forms involve the unborn or newborn child, causing premature birth, death, or serious brain damage and blindness. In a chronic form in adults, the infection may also cause eye disease leading to blindness--this is probably the most important manifestation of the infection.

The major unanswered problem of toxoplasmosis is how the disease is transmitted to man. Because of the widespread occurrence of the parasite in many species of animals and birds--dogs, cats, swine, sheep, cattle, chickens and pigeons--attention has been directed to a number of these as sources from which human infections may be derived, through consumption of animal food products or by insect transmission.

CAUSATIVE AGENT OF TOXOPLASMOSIS FOUND IN PORK

The causative agent of toxoplasmosis, a microscopic protozoan parasite, was demonstrated for the first time in pork samples, obtained from an Eastern city abattoir, by Dr. Leon Jacobs and Miss Marjorie L. Melton, NIAID scientists of the Laboratory of Tropical Diseases.

Toxoplasma, the parasite, was first found in swine in Ohio in 1954, but the presence of parasites in the muscle of the animals, which is the crucial point in relation to transmission to human beings, had never been investigated. Laboratory studies show that animal species differ in their ability to harbor parasites in muscle, and it was necessary to devise a technique which would allow the sampling of large pieces of tissue. Small pieces of tissue may frequently be reported negative because of uneven distribution of the parasites.

The investigators tested 50 pork samples for the parasite, using a simple digestion technique which they had developed. The meat was digested for 2 hours, using a solution of hydrochloric acid, salt, water, and pepsin. Groups of 10 or more mice were injected with the suspension from each of 50 samples.

The majority of the mice in 8 of the 50 groups died of toxoplasmosis in 7 to 14 days. The mice of the remaining groups were tested serologically by the dye test and 4 additional positive groups were found.

Laboratory studies on temperatures which the toxoplasma parasites in the flesh of animals can resist indicated that thorough cooking of pork or other meats will kill the parasites.

The newly developed digestion technique, the scientists say, will now allow extensive epidemiological studies on toxoplasmosis not heretofore possible.

ACQUIRED TOXOPLASMOSIS TREATED WITH PYRIMETHAMINE AND TRIPLE SULFONAMIDES

The results of joint studies of the Laboratory of Clinical Investigation and the Laboratory of Tropical Diseases indicate that patients with acquired toxoplasmosis can be treated effectively with pyrimethamine and triple sulfonamides.

These drugs were tested first in experimentally infected mice and rats by the Laboratory of Tropical Diseases.

The patients in these studies represent the second and third cases of acquired toxoplasmosis treated with these drugs. One of the patients had contracted a toxoplasmic infection while working in a research laboratory, and the other had a disease suggestive of leukemia and later developed chorioretinitis.

Both patients responded promptly to this therapy, with decrease in the size of lymph nodes and drop in antibody levels. The skin tests for toxoplasmosis remained negative for more than 60 weeks after the onset of symptoms.

In the laboratory-acquired case, the fever and skin rash quickly subsided. Two months after treatment had been completed, lymph node enlargement recurred and antibody levels increased. The patient became well without further treatment, and has remained in good health for the past 18 months.

The eye lesion in the patient with chorioretinitis showed a gradual decrease in size, with eventual healing and scar formation.

One of the patients represents the first parasitologically proved case reported in whom a chorioretinal lesion developed in the acute or subacute stage of the disease.

ANTIBIOTICS

The increasing number of antibiotics available for treatment of infectious diseases and their widespread use in medical practice today have created many complex problems. The organisms which cause these diseases vary widely in their susceptibility and resistance to antibiotics and the selection of the most effective drug therapy requires a careful evaluation. The mode of action of antibiotics in the intestines--their growth-promoting effect and concentration--is not clearly understood.

SINGLE AND COMBINED ANTIBIOTIC ACTIVITY AGAINST STAPHYLOCOCCI

The increasing resistance of staphylococcal infections to antibiotics has been under investigation by Dr. Maxwell Finland, an NIAID grantee at the Boston City Hospital, for a number of years. Dr. Finland recently completed tests comparing the antibacterial activity of erythromycin, oleandomycin, spiramycin, tetracycline, and combinations of each of the first three with tetracycline, against common strains of staphylococci.

The tests employed the antibiotics in agar and in blood plasma against staphylococci. The results indicated that erythromycin used alone is superior in activity to oleandomycin or spiramycin alone, erythromycin-tetracycline combination produced better antibacterial activity than either of the other two antimicrobials combined with tetracycline, and adding tetracycline to mixtures did not increase activity against tetracycline-resistant strains.

Dr. Finland in summarizing his findings said, "Oleandomycin and spiramycin are sufficiently inferior to erythromycin to indicate that their adoption for general use in the treatment of infections is unwarranted and should be discouraged. . . The distinct superiority of erythromycin over the others against the greatest majority of pathogens suggests it remain the antibiotic of choice in this group . . .

"The introduction and advocacy of the use of antibiotic combinations of the type dealt with in these studies, particularly the combination of tetracycline with either oleandomycin or spiramycin, and especially in fixed ratio, is not justified by available data, represents bad practice, and is not in the best interest of the patient."

The dangers of combined antibiotic therapy, Dr. Finland cautioned, are inadequate therapy through fixed combinations, increased hypersensitivity and toxicity by using two drugs, and the development of bacteria resistant to either or both of the drugs. Predetermined combinations in fixed ratios, he added, should be scrupulously avoided in the treatment of a serious infection, such as bacterial endocarditis.

ORAL PENICILLIN EFFECTIVE IN TREATMENT OF STREPTOCOCCAL ENDOCARDITIS

Streptococcal endocarditis, an inflammation of the membranous lining of the heart and valves, was effectively treated by large oral doses of penicillin V in conjunction with streptomycin and dihydrostreptomycin. Dr. David E. Rogers and associates at New York University-Cornell Medical Center in New York City conducted this clinical study, which was supported by an NIAID grant.

This investigation was initiated to provide a better method of administering penicillin than the usual intramuscular administration of the drug which causes complications due to the high dosage therapy required in the treatment of this disease.

Penicillin V, orally administered, was well tolerated by 4 of 6 patients, and there was no evidence of the blood cell formation being affected, or liver or kidney damage, and no allergic symptoms occurred as a result of the treatment. Increasing nausea and subsequent vomiting developed in two patients and were difficult to control without withdrawal of penicillin by the oral route.

All patients received at least 4 weeks of penicillin therapy, 2 million units of penicillin V by mouth every 4 hours. One gram of streptomycin or dihydrostreptomycin was alternately administered intramuscularly every 12 hours.

In general, subjective improvement was noted within 2 or 3 days of initiation of therapy. Clinical and bacteriologic cures were obtained in all patients with no signs of relapse on subsequent follow-up periods from 3 to 10 months.

The results obtained with these patients, the investigators say, demonstrate that penicillin-sensitive streptococcal endocarditis can be satisfactorily treated with large doses of penicillin V given in conjunction with streptomycin and dihydrostreptomycin. They caution that in using this oral therapy, serum penicillin levels must be determined frequently to assure that the therapy is adequate.

GROWTH-PROMOTING EFFECT AND INTESTINAL CONCENTRATION OF ANTIBIOTICS IN THE RAT

A study of the mode of action of antibiotics in the rat by Drs. K. R. Johansson and Elliot C. Dick of the University of Minnesota showed that little relationship exists between the growth-promoting effect of antibiotics and the level of intestinal concentration of the drug.

The antibiotics tested included bacitracin, aureomycin, terramycin, procaine penicillin G, potassium penicillin G, magnamycin, and inactivated procaine penicillin and aureomycin. The drugs were administered orally, parenterally, and intraperitoneally.

Bacitracin enhanced growth markedly when injected parenterally, but insignificantly when fed to rats in their rations. This finding the investigators considered particularly remarkable since bacitracin is very poorly absorbed from the intestinal tract and no antibiotic activity could be detected in the intestines of animals receiving this drug parenterally.

Orally and parenterally administered aureomycin promoted growth significantly, whereas terramycin was effective only when given orally or intraperitoneally. A very high level of antibiotic activity was present in rats given these drugs by mouth, while less was found when these substances were injected parenterally.

The growth-increasing effects of penicillin varied according to the salt employed. Procaine penicillin stimulated growth when given orally or intraperitoneally, while potassium penicillin was stimulatory only when given orally. This inconsistency, the investigators believe, probably lies in the differing capabilities of the two salts for attaining long-lasting levels after injection.

Magnamycin, when injected intraperitoneally, was highly stimulatory to growth without producing detectable alterations in the intestinal microflora.

Inactivated antibiotics did not stimulate growth significantly but a very substantial amount of antibiotic activity was found in the intestines of rats fed alkali-inactivated aureomycin.

TISSUE CULTURE

Life in a test tube--the tissue culture system of growing cells in nutrient solutions under glass where they may multiply and organize themselves into sheets of living tissue--has made possible broader studies of many life processes. The widening scope of research on the adenoviruses and others of the so-called latent viruses, stems, for example, from the development by NIAID's Laboratory of Infectious Diseases of new techniques for "unmasking" agents of this type in tissue cultures. In essence, this process for unmasking viruses involves placing the selected tissues in tissue culture for prolonged periods: several weeks as compared with the few days needed to get most cell lines to grow out. The longer period is significant because any antibodies present in the tissues are gradually washed out when maintenance culture fluids are changed periodically. This means that the substances which normally serve to inhibit virus multiplication are removed, enabling the viruses to proliferate to the point where their presence may be detected by the cell destruction they produce. In studies of cellular biology, also, tissue culture is a tool which greatly extends the research potential.

TISSUE CULTURE PROMISING AS SCREEN FOR CHEMICALS TOXIC TO CANCER

Dr. Harry Eagle of NIAID's Laboratory of Infectious Diseases is testing the practicability of using tissue cultures in screening for chemicals toxic to cancer. If the promise of initial trial screenings with over 200 chemical compounds is borne out, the tissue culture method would be faster and more economical than animal testing.

Initial results suggest that the majority of anti-cancer agents now available may owe their activity to direct toxic action in animals since 70 percent of the compounds with known anti-cancer action in animals proved also to be toxic to the cells in test tubes.

Many compounds with anti-tumor effects in animals were toxic in culture even when they were introduced in a one-to-ten million dilution. A toxic reaction in the test tube at this small concentration almost always meant that the substance was also poisonous to tumor cells in the living animal.

Paradoxically, no special susceptibility of cancer cells over normal cells was indicated in any of the tests even with agents known to have a partial selective action on tumors in animals.

Dr. George E. Foley of the Children's Cancer Research Foundation and the Department of Pathology, Harvard Medical School cooperated in this research.

ANTICELLULAR SERUM STUDY MAY YIELD INFORMATION ON VIRUS-CELL RELATIONSHIPS

Laboratory workers adding serums to test tube cell cultures in which viruses are studied sometimes find a serum interferes with the growth of various viruses--not just with the type against which it contains antibodies. It is nonspecific. Dr. Karl Habel and associates of NIAID's Laboratory of Infectious Diseases are turning this mysterious and disturbing phenomenon to good purpose: it may provide information on how viruses impel cells to support virus reproduction at the expense of normal cell functions--information basic to advances against viral diseases.

The NIAID scientists show how this kind of serum obtains its effect and hypothesize on its mode action. The effect is acquired during hyperimmunization. To build up a great amount of antibodies against a certain virus, repeated injections of viral materials may be given an animal. Eventually, serum withdrawn is hyperimmune and contains vast concentrations of specific antivirus bodies. But the injections also contain minute quantities of tissue material. The animal blood forms heavy concentrations of antibodies against these tissue cell proteins, too. It becomes anticellular as well as antiviral. Blood serum taken before immunization of a rabbit, for example, has been devoid of effect, while increasing virus-inhibiting effect has been produced with sera withdrawn serially in the course of immunization. Apparently hyperimmune sera may contain not only (1) antibodies that couple with and immobilize the specific kind of virus against which they were formed but also (2) antibodies that couple with some essential protein common to many kinds of cells and that block various viruses from using this for their reproduction. Thus, anticellular sera are seen to inhibit various viruses in various kinds of cells.

Hypotheses on the mode of action of "anticellular" sera present a number of experimental challenges new to virus-cell research. Observations that the type of cell in which the virus is produced has no influence on the inhibitory phenomenon; that human red blood cells can adsorb out effective antibodies; that only certain viruses are inhibited; that antisera can be prepared against specific cell fractions; and that inhibition can occur even after the virus theoretically has penetrated beyond the cell surface suggest further exploration of anticellular sera phenomena may lead to a better understanding of basic virus-cell relationships of broad significance.

BIOSYNTHETIC MECHANISM COMPARED IN NEW CELL LINE AND ONE CULTURED FOR YEARS

As cells reproduce themselves repeatedly for months and years in test tube tissue cultures, they become far removed in time from their original environment in the living animal. The question is sometimes raised as to whether certain experimental results are due merely to this artifact.

During recent studies, Dr. Harry Eagle and associates, Laboratory of Infectious Diseases, NIAID, showed that, although 8 amino acids are known to suffice for nitrogen balance in man, a number of cultured human and lower animal cell lines were found to require 13 amino acids for survival and growth. One explanation for the anomalous and uniform requirement for at least 13 amino acids was that the cell strains had altered in the course of their prolonged cultivation, to the degree that normal biosynthetic mechanisms leading to the formation of 5 additional amino acids were no longer operative.

The present experiments were undertaken to explore this possibility by determining the amino acid requirements of monkey kidney cells in their first culture passage, prior to extensive proliferation.

Trypsinized suspensions of monkey kidney (MK) cells were obtained from three different laboratories. Modified Earle-Highhouse T-15 flasks were inoculated with 300,000 to 1,500,000 cells. These were tested within 24 hours after their removal from the animal hosts. They were found to require the same 13 amino acids for survival and growth as cell lines serially propagated in culture for years. Apparently the requirement for additional amino acids is not an artifact.

Among other observations: the MK cultures differed from serially propagated cell lines in their capacity to synthesize glutamine from low concentrations of glutamic acid; glycine was growth stimulatory for MK cells in primary culture; and cells grown in a glycine-deficient medium usually failed to survive subculture.

RABIES

As many as 50,000 individuals receive antirabies prophylaxis each year. Rabies vaccine, in spite of continuing efforts to improve it, remains a crude product. One of every few thousand individuals given the course of immunization may be paralyzed by foreign matter inherent to this vaccine. While this paralysis is not always severe, the hazard underscores the importance of studies under way to improve methods of rabies immunization.

VARIOUS SCHEDULES OF ANTISERUM AND/OR VACCINE EVALUATED IN RABIES

When 27 individuals in a large group traveling in the mountains of Iran were bitten by a rabid wolf, serum containing antibodies against the rabies virus was given to some, as well as vaccine. Antiserum plus vaccine showed promise in this trial.

Scientists from several agencies have since cooperated in evaluations of different schedules of serum and vaccine inoculations. In addition to Dr. Karl Habel of NIAID and Dr. J. P. Fox of Tulane University, who has conducted other developmental experiments with vaccines under NIAID grant support, the cooperating scientists are from the World Health Organization; the PHS Communicable Disease Center; the Pasteur Institutes of Paris and Iran; the American Cyanamid Company; and government laboratories in Spain and Israel.

Studies are reported of the various blood levels of antibodies produced by schedules of phenolized inactivated vaccine given subcutaneously and high egg passage (HEP) Flury strain vaccine given intradermally, with and without inoculation of antirabies serum. The volunteers, previously unexposed to rabies and with no history of rabies vaccination, were followed for periods up to 60 days.

When antirabies serum was given in a single inoculation, the recipient gained "ready-made" or passive antibodies that persisted in his bloodstream for as long as 42 days. In addition, 14 daily doses of phenolized vaccine stimulated the recipient to manufacture his own protective antirabies particles. However, two inoculations of antirabies serum not only failed to increase the level of passive antibodies, but actually interfered with active antibody production when vaccine was given.

The investigators also find that three inoculations of phenolized vaccine given five days apart cannot be substituted for the 14 daily inoculations, which must still be considered a minimum for immunization after exposure.

Prior to exposure, however, three inoculations of HEP Flury vaccine at five-day intervals gave lasting protection if followed by a booster dose six months later. This schedule might be satisfactory for pre-exposure immunization of veterinarians, dog-catchers, and other high risk groups.

WILDLIFE DEVOUR RABID BATS; STUDIED AS FACTOR IN RABIES INFECTION CHAIN

NIAID grantees J. V. Irons and R. B. Eads of the Texas State Department of Health--concerned because bat rabies might be transmitted to wild animals, to domestic animals, and directly to humans--have studied the role of bats in the basic infection chain of rabies in wild life. Their work suggests that the association between bats and carnivores may be a more important factor in maintaining reservoirs of rabies virus than had been recognized.

"That the opportunity exists to transmit the disease to other wild animals is evident. We have located and investigated 9 caves in Central Texas in which multimillion colonies of the free-tailed bat (Tadarida mexicana) maintain summer occupancy. It has been noted that the population levels of raccoons, skunks and foxes is considerably higher in the vicinity of these caves... The frequency with which raccoon fecal droppings around the caves contain bat remains indicates that bats are important dietary items for these animals... Whether or not inter-species spread of rabies is of significance is not known, but this Department has demonstrated natural infection in a wide range of animals."

Thirty-five people have died in Texas of rabies in the last 12 years. An estimated 5,000 multi-injection human rabies treatments are administered annually. A number of foxes, skunks, wolves, bobcats, oppossums, raccoons, and coyotes are demonstrated to be rabid each year by laboratory confirmed tests which also are positive for approximately 1,000 dogs and 60 cats yearly. Livestock are also involved.

Twenty-two cases of human bat bites were reported in Texas during 1954-56. Fifteen of the bats involved were tested, and 5 were proven rabid. Two of the positives were T. mexicana and three were Lasiurus borealis bats. The grantees report upon two recent human deaths attributable to bat exposure in Texas. One of these was a member of their team investigating the bat rabies problem. He presumably acquired the infection while handling the animals.

FUNGUS DISEASES

The fungus diseases of man (mycoses) vary in severity from relatively mild skin infections such as ringworm to systemic infections which are reported in the United States to be the cause of several hundred deaths each year. In recent years fungus diseases are more frequently suspected and diagnosed than ever before.

AMPHOTERICIN EFFECTIVE AGAINST HISTOPLASMOSIS, OTHER SYSTEMIC FUNGI

Amphotericin B has been shown to be an extremely effective antifungal agent in animals infected experimentally with several different disease-causing fungi. Dr. Donald B. Louria of NIAID's Laboratory of Clinical Investigation studied blood levels of the drug

in volunteer patients. Amphotericin given by mouth was absorbed poorly, but blood levels of the same drug given intravenously were consistently high, except in the areas of the central nervous system.

A report of clinical observations on the use of various forms of amphotericin in patients with systemic fungal diseases supported these findings. Dr. John P. Utz, also of the Laboratory of Clinical Investigation, found amphotericin promising in the treatment of some systemic fungi. When given oral doses, three patients with cryptococcal meningitis reported subjective improvement; one patient with disseminated histoplasmosis showed marked objective signs of improvement, but subsequently died of his disease; and one patient with cryptococcosis of soft tissues and bone made an apparent recovery. On intravenous amphotericin, one patient with disseminated histoplasmosis apparently recovered, and two patients with blastomycosis were benefited. NIAID clinicians feel that, at the present state of knowledge, the use of this drug should be regarded as experimental.

HISTOPLASMOSIS ENDEMIC IN EASTERN AS WELL AS IN CENTRAL UNITED STATES

Histoplasmosis is characterized by mild to severe lung involvement, lesions, or other illness. When seen on X-rays, lung spots may be misdiagnosed as tuberculosis. Histoplasmosis is apparently more highly endemic in Washington, D. C., and in Virginia, Maryland and nearby Pennsylvania than has been generally recognized.

Dr. Chester W. Emmons, NIAID Laboratory of Infectious Diseases, and Charlotte C. Campbell, Walter Reed Army Institute of Research, have assembled evidence of the prevalence of the mold infection based on studies of sporadic and individual cases observed in civilian and military hospitals in this region and of environmental exposures in these cases. Histoplasma capsulatum was isolated from soils of 22 premises throughout this area. All but two of these soils had been fertilized with chicken manure. In many of the 52 culturally verified or serologically presumptive cases of histoplasmosis reported, the patients had been exposed to these soils. The investigators suggest there is no longer reason to continue to link the acquisition of histoplasmosis with residence or travel in the central United States; it is endemic also in the East.

PASSIVE IMMUNITY FOUND IN NEWBORN OF MOTHER WITH CHRONIC HISTOPLASMOSIS

In a grant-supported study, Dr. L. D. Zeidberg of the Tennessee Department of Public Health and Vanderbilt University School of Medicine, Nashville, has found the first evidence that histoplasmosis antibodies may be transmitted by a mother to her newborn infant. The opportunity to study placental transfer of fungal antibodies

is rare, since the disease is more prevalent in males than in females.

The investigators studied the transmission of antibodies to two infants of a mother with chronic cavitary pulmonary histoplasmosis. One child was a full-term boy of normal weight and the other was a premature girl. These children were born about a year apart. At the time of both deliveries, tests for histoplasmosis were made of the placentas, mother's blood, and the cord bloods. The placentas were negative for H. capsulatum, the mother's blood showed a histoplasmosis complement-fixation titer of 1:80, and the cord blood of the full-term infant was the same as the mother's, while the premature infant had a titer of 1:40 compared with the mother's titer of 1:80. It has been shown that a significant direct correlation exists between the birth weight and the placental permeability and that passive immunity is lost at about the same rate by premature and normal infants.

"The presence of circulating antibodies in the cord blood at birth," the researchers explain, " may mean either that the infant has congenital disease or that a passive transfer of antibodies from mother to newborn has occurred. Ordinarily the presence of circulating antibodies in the absence of skin sensitivity is indicative of the passive placental transfer of the antibodies. Proof of this becomes available as serum titers decline and disappear altogether within a few months after birth, as was the case in both of the infants in this study."

The passive protection resulting from transplacental acquisition of antibodies, they point out, may mean the difference between life and death for an infant born of a mother with the disease. Histoplasmosis in infancy is a highly fatal disease with a mortality rate of almost 100 percent.

FUNGI INFECT HEART LINING: RARE DISORDER STUDIED AT CLINICAL CENTER

Doctors R. K. Merchant, D. B. Louria, and J. P. Utz of NIAID's Laboratory of Clinical Investigation and Doctors P. H. Geisler and J. H. Edgcomb of the Pathological Anatomy Department of the Clinical Center have contributed to knowledge of a rare fungus disorder of the heart, fungal endocarditis. In the majority of cases of this disease severe inflammation and deterioration of the heart lining occurs, resembling bacterial infection in this area. On the basis of their studies, including autopsies of Clinical Center cases (two caused by Histoplasma capsulatum and one by Coccidioides immitis), the investigators suggest serious consideration of the possibility of fungal endocarditis when the patient has known systemic fungal disease and shows physical signs of endocarditis, such as a heart murmur or evidence of major emboli; and when the patient presents a clinical picture of subacute bacterial endocarditis but routine blood cultures are sterile and there is no obvious evidence of fungus infection. In the latter case, an intensive search should be made in blood, urine, bone marrow, lymph nodes and elsewhere for evidence of systemic fungal infection; if found, it may be an important clue to the nature of the endocarditis process.

Fungal infections seem to be increasingly common as the extensive use of antibiotics alters the flora of respiratory and intestinal tracts. As the therapy of fungal diseases is developed to greater effectiveness, diagnosis of this type infection will be increasingly important. Awareness of fungal endocarditis and the use of cultural and histological techniques described by the investigators will allow diagnosis of fungal endocarditis more frequently during life rather than at autopsy.

MUCORMYCOSIS NEW DISEASE AS "HARMLESS" FUNGI INVADE, RIDDLE ARTERIES

In mucormycosis--a new disease in the United States--certain fungi, usually harmless and common in the environment, may emerge as pathogens with unique affinity for the arteries. Fungi-riddled arteries soon manifest ocular, cerebral, pulmonary, intestinal or disseminated forms of disease. Blindness or fatal cerebral strokes are among the complications. The acute rather than chronic nature of the fungus invasion is atypical.

This picture of the disease (most frequent agent: Rhizopus fungus) is presented by NIAID grantee, Dr. Roger D. Baker, a pathologist associated with Duke University School of Medicine and Chief of the Laboratory Service, Veterans Administration Hospital, Durham, N. C. Dr. Baker, who has conducted laboratory research on mucormycosis, comments: "The disease occurs more frequently than is realized (is probably world-wide), and without biopsy or autopsy diagnosis might be overlooked. Therefore, physicians will wish to know of this fungus infection and of the circumstances under which it develops."

Mucormycosis is usually encountered as a complication of other diseases, of which diabetes mellitus and leukemia are the commonest, but multiple myeloma, fatal burns, and cirrhosis are other antecedent conditions. Cortisone, corticotropin (ACTH), antileukemic chemical agents, as well as antibiotics may be other predisposing factors. The improvement in the control of bacterial diseases by the use of antibiotics may explain why mucormycosis is now encountered. Diabetics who formerly developed staphylococcic infections, for example, may now contract this new fungus infection. The fungi enter the nose, produce sinusitis and orbital cellulitis. They penetrate artery walls, later invade veins and lymphatics. Diagnosis depends on recognition of the fungus in specimens of tissue or of body fluids. Since Rhizopus fungus is a common contaminant in laboratory cultures, however, recovery of this organism in culture is not in itself diagnostic. Treatment consists in controlling the diabetes if present and discontinuing the use of the suspected drugs.

OTHER RESEARCH

ADENOVIRUSES IMPORTANT AS CAUSE OF EYE DISEASE; IRRITATION MAY POTENTIATE

In an area of research conducted cooperatively with the National Institute of Neurological Diseases and Blindness, Dr. Robert J. Huebner and Associates of NIAID's Laboratory of Infectious Diseases report increasing evidence that a significant amount of eye disease is caused by adenoviruses. Irritations such as rubbing the eyes or contact with water or chlorine while swimming may potentiate the viruses.

The investigators comment that "despite the attention given to adenoviruses as a cause of respiratory disease, their role in the etiology of ocular disease may well turn out to be equally if not more important." As a result of a number of surveys, certain adenoviruses, namely types 3, 7-prime, and 8 are now well established as causes of ocular disease. Other serotypes have been recovered from occasional cases of conjunctivitis and have produced the disease in volunteers. Four new types have been found only in the conjunctiva of children suffering from eye disease in Saudi Arabia. (Work of Murray & Chang, et al, Harvard.)

The growing importance of adenoviruses to opthalmology does not, however, discount their importance as a cause of feverish, grippe-like illnesses. Vaccines developed by NIAID against these respiratory diseases have been shown to be sufficiently effective in military recruits so that it is now possible, the investigators believe, to predict that commercially produced vaccines will soon be in general use in those population groups known to suffer high rates of respiratory diseases due to adenoviruses.

NEW FINDINGS ABOUT MUMPS MAY REVISE CONCEPT OF PERIOD OF COMMUNICABILITY

Research by Dr. John P. Utz and associates of NIAID's Laboratory of Clinical Investigation has challenged some old concepts about mumps. Using new techniques for isolating the virus, these scientists found that the disease may be communicable longer than has been thought.

By isolating the mumps virus for the first time from urine during intensive studies of 21 patients with apparent mumps infection, the investigators were able to demonstrate that the infectious virus particles are present in the body waste for as long as 13 days after onset of illness. This may cause revision of the present concept of the period of communicability of mumps. Generally, this period has been thought to be from about two days before distinctive symptoms, and persisting as much as nine days thereafter, but no longer than the swelling of a salivary gland.

The presence of gross amounts of virus in the urine, although blood specimens failed to yield virus, also raised the possibility of the growth of virus in the kidneys. Current studies by the Laboratory of Clinical Investigation are designed to investigate whether kidney damage may occur in mumps.

RUSSIAN "POLIOVIRUS TYPE 4" IDENTIFIED AS A COXSACKIE; MIGHT CAUSE PARALYSIS

Three broad types of poliovirus have been isolated. Recently, however, Russian workers (Chumakov, et al) reported that they had isolated a "poliovirus type 4" from stool specimens of acutely paralyzed children. This virus has now been shown by Dr. Karl Habel, NIAID Laboratory of Infectious Diseases and Dr. Ladd N. Loomis, NIAMD Laboratory of Pathology and Histochemistry to belong instead to the Coxsackie A7 virus strain. Habel and Loomis comment that the mistaken identification by the foreign scientists was understandable since they did not have specific Coxsackie virus typing sera available at the time.

The NIH investigators confirm the Russian results insofar as demonstrating that their A7 virus is capable of producing polio-like lesions in the monkey central nervous system, and have further demonstrated this to be true of 2 strains of A7 virus isolated directly from the stools of children in the United States with aseptic meningitis. Since this is the only virus type found to produce such polio-like lesions in the monkey, the analogy is suggested that Coxsackie A7 might cause paralytic lesions resembling poliomyelitis in humans. However, no virus other than the 3 types of poliovirus has, to date, been proven a cause of paralytic polio. An ever-growing number of viruses other than poliovirus, however, are shown to produce a syndrome sometimes mistakenly diagnosed as nonparalytic poliomyelitis.

The NIH scientists suggest that more complete investigations of "paralytic poliomyelitis" in larger numbers of individuals will probably result in more reports of stools and serological tests that do not indicate a poliovirus infection. Such paralytic cases have already been found. The hypothesis that Coxsackie A7 may be causing an occasional "paralytic polio" case is further strengthened by the fact that the virus was isolated by the Russians from cases resembling acute paralytic poliomyelitis in children.

Although it has many poliovirus properties the Russian virus is a typical Coxsackie A, and is immunologically distinct from the 3 known poliovirus types; thus, a vaccine effective against the latter agents would not minimize the effects of the Coxsackie virus infection.

AGE, ENVIRONMENT FACTORS AS RADIATION AFFECTS HOST-VIRUS REACTIONS

In a study of the tobacco mosaic host-virus system, NIAID grantees Albert Siegel and Sam G. Wildman of the University of California at Los Angeles are seeking to gain a more comprehensive understanding of how radiation affects a biological material. This work concerns the properties of nucleic acid, which is the core of the virus particle and apparently responsible for initiating the process of infection. The rest of the virus, 95 percent of the total, is made up of protein.

Recent findings in this study have shown that the nucleic acid of certain strains of tobacco mosaic virus is protected against inactivation by ultraviolet light by combination with virus protein. When nucleic acid alone is used to initiate infection, the processes of infection begin immediately. This is in contrast to intact tobacco mosaic virus in which a delay of several hours is encountered while the nucleic acid of the infecting virus particle is released from its surrounding protein coat.

In working with tobacco mosaic strains varying five-fold in radiation sensitivity, the investigators found that the nucleic acid preparations derived from those strains were equally sensitive in the absence of the protein. This suggests that the union of nucleic acid with protein in resistant strains results in a virus particle more difficult to rupture after subjection to ultraviolet radiation, or more easily repaired, than that found in sensitive strains.

In another aspect of the tobacco mosaic host-virus study, it was shown that plant leaves above the source of infection, which had completed their expansion prior to the start of infection, permit only slight amounts of virus formation. Leaves in the process of expansion, however, permit extensive virus formation. Often this amount is greater than the amount of virus that can be extracted from the directly inoculated leaf. The difference in virus content between a fully-expanded, and the young expanding leaf may be more than ten-fold.

These results point to the profound influence of age and physiological status of the host as conditions governing the rate and extent of virus multiplication. While many new approaches are emerging with respect to the general problem of relating symptom expression to the physiological malfunctioning of the host resulting from virus infection, the investigators point out the future experimentation will require a more precise environmental control of the host-virus system than is now available.

TWO YEAR STUDY IN HOSPITAL FOLLOWS BACTERIAL DIARRHEA IN 2865 INFANTS, CHILDREN

Diarrheal infections in infants and children are an important disease problem in the United States and all other countries of the world. Outbreaks of these infections can be particularly serious in hospital nurseries and children's wards. To determine their prevalence, the number of deaths resulting from these infections, and the role played by convalescent carriers, a two-year clinical study was made by Dr. Merlin Cooper, NIAID grantee at the Children's Hospital in Cincinnati.

Of the 2,865 patients tested for bacterial infections due to salmonella, shigella, and enteropathogenic E. coli, isolations of the latter organism exceeded the combined total of the other two. E. coli and salmonella organisms were found to be more prevalent in children in the first year of life, while shigella had its peak incidence in the second year age group.

Diarrhea was associated with all three microbial infections in a comparable degree. A large number of patients with diarrhea, however, had negative cultures for salmonella, shigella, and E. coli.

Successive and/or multiple infections with the three organisms occurred in a small number of patients. Infection with one serotype of E. coli did not protect against subsequent infection with another serotype.

The mortality rate for this study was low. Three deaths occurred in patients with E. coli infections and one with salmonella. There were no deaths in the group of patients from whom the shigellae were isolated.

Specimens were obtained from convalescent patients one to two weeks after therapy had been discontinued to test for carriers of infections. The extremely high convalescent carrier rate--36 percent--among patients from whom salmonellae had been isolated indicates the need for better antibiotic therapy.

BACTERIA TRACE PATHWAY OF URINARY-TRACT INFECTION PAST INLYING CATHETER

Urologists report that operations involving the urinary tract are increasing, as are the numbers of people entering the ages when such procedures are more frequently required. Fortunately, the practice of preparing an old person for surgery carefully and the general high competency of the modern surgical team have contributed to an excellent prognosis, even when octogenarians are accepted for surgery.

However, a remaining hazard is infection attendant upon the simple use of an inlying catheter introduced into the bladder through the urethra for the withdrawal of urine.

Dr. Edward H. Kass and associates, NIAID grantees at Harvard and Boston City Hospital, report upon experiments to elucidate the pathway of infection by introducing bacteria on surfaces near the urethra in three patients with inlying catheters. A strain of Serratia marcescens was used. While nonpathogenic to man, it is related to the coliform organisms

usually found in urinary tract infections. The bacteria were applied lightly to the surfaces without touching the catheter. On the third or fourth day culture of urine specimens yielded many thousands of S. marcescens per milliliter. Although the precise pathway is unproved, a possible route of entry may be the fluid-filled, thin space between the catheter and the urethral mucosa. The fluid here provides an excellent culture medium in which certain bacteria could multiply and spread from the exterior to the interior of the urinary tract. Brownian forces--the vibratory movements of microscopic particles suspended in a fluid--serve to distribute even nonmotile, unclumped bacteria and might account for the spread of the bacteria from the tissue surrounding the catheter "pipeline" back to a point where, while proliferating, the organisms could be excreted in quantity in the urine.

VIRUS-LIKE AGENT OPENS WAY FOR LISTERIOSIS AGENT IN FATAL BRAIN DISEASE

A virus-like agent which enhances the activity of Listeriosis bacteria (cause of central nervous system disease in man and animals) has been found by NIAID grantee Carl Olson at the University of Wisconsin. Naturally occurring seasonal epizootics of Listeriosis in sheep and cattle provide the study material.

A type of infection resembling Listeriosis in newborn infants was observed in sheep experimentally infected with Listeria bacteria and with LEA--the Listeria enhancing agent. This agent was first isolated from feverish animals where the bacteria was not present, and later from bacteria-infected sheep in a natural outbreak of Listeriosis.

In a search for other agents which might also enhance the disease-producing activity of the Listeria bacteria, an isolate from mucosal disease--a fatal disease of young cattle--was found to have a similar function.

A number of experiments indicated that Listeria plus LEA is more pathogenic than the bacteria alone. Disease developed, for example, in nearly 100 percent of sheep given the combination intravenously as compared with 25 percent given Listeria alone. When the enhancing viral-like agent is mixed with Listeria and sprayed into the nose, a fatal encephalitis will develop in about three weeks. Listeria organisms alone sprayed into the nasal cavity of test sheep will only rarely gain access to the brain.

Besides developing information about the mechanism by which Listeria gains access to the brains of animals (usually causing fatal encephalitis) the research should have significance in the broader area of microorganism relationships in disease, a field of growing interest to which germ-free research has brought a new investigative tool.

FATAL, DEGENERATIVE DISEASE OBSERVED IN CHILDREN DESCRIBED AS NEW ENTITY

A "relentless and malignant" disease of childhood has been recognized as an entity for the first time in an NIAID grant-supported study by Dr. Robert A. Good at the University of Minnesota. The syndrome consists of chronic inflammation of the lymph nodes, pulmonary infiltrations, enlargement of the liver and spleen, and a chronic eczema-like inflammation about the eyes, nose and mouth. Excessive gamma globulin is also present without correlation to degree of severity. The fine network of tissues which encompasses such organs as the lymph nodes, liver, and kidneys apparently degenerates. The investigators comment that the disease has a striking resemblance to an infectious process, but no known bacterium, fungus, or virus was found as a primary agent. Allergic factors were not demonstrated.

Death occurred in three of the four male patients observed at the university hospital. The fourth was in an early stage of the illness. Three of the patients were under one year of age at time of admission and the fourth was two years old. Empirical therapy was ineffective. This included X-ray; extensive use of antibiotics, singly and in combinations; blood and plasma transfusions; and injections of gamma globulin.

The epidemiology of this disease is not known. The Minnesota investigators have revealed it as a new entity and have characterized the problem for continued studies.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

ARTHRITIS AND METABOLIC DISEASES

1957

Items of Interest on Program Developments and Research Studies Conducted and Supported by the National Institute of Arthritis and Metabolic Diseases

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The broad areas of medical research covered by the programs of the National Institute of Arthritis and Metabolic Diseases involve some of the most complex and varied problems facing science today.

To solve these problems, even to attack them intelligently, requires a diversity of skills, a planned approach combining many talents and techniques. More and more, it has been found, the research team, combining the ideas of scientists schooled in several different disciplines, has been effective in finding answers to formerly insoluble problems.

An example is afforded by the work of just such a team, which as reported herein, discovered the cause of a relatively rare metabolic disease. Biochemist, clinical investigator, pathologist, and surgeon combined their skills, using radioactive isotopes and electronic instruments devised by physicists and new techniques which resulted from the cross-fertilization of ideas and the blending of their separate skills.

Similarly, among other advances recorded in this report, most are the result of research teamwork and of the hybridized approach.

A few selected accomplishments of the past year are reported on the following pages.

Prepared, January 1958

DIABETES

The diabetic walks a narrow path between two hazards--diabetic coma on one side, hypoglycemic shock on the other. With the aid of drugs, proper diet, and good medical care, the diabetic today can traverse this path with a fair degree of ease and confidence. Yesterday, before insulin, the disease proceeded almost inevitably into acidosis, coma, and death. As for tomorrow, the ultimate goals--prevention and cure--will be reached when new knowledge of the vital metabolic processes becomes available. Progress towards this end is being made.

Daily advances augur well for the two million diabetics in this country. During the past year, for example, a new form of treatment was made available upon prescription to the general public. Tolbutamide (Orinase), a substitute for insulin in tablet form, seems to control diabetes in certain types of cases. It provides a welcome simplification for those it can help--medication by mouth instead of by injection.

The National Institute of Arthritis and Metabolic Diseases has developed a threefold research attack upon diabetes, both in its own facilities and through its grants program. The Institute conducts and supports immediately applicable clinical investigations and fundamental studies of the disease process itself. Through its training grants, it is developing research talent and clinical manpower needed to carry on and intensify these efforts in the future.

NEW ORAL ANTIDIABETIC DRUG NOW IN COMMON USE

Although scientists still have not figured out exactly how it achieves its effects, tolbutamide (Orinase), the new oral antidiabetic drug, is now in common use. One out of every four diabetics, with advice from their physicians, have switched from insulin by injection to the new tablets, according to recent informed estimates.

Two years of intensive research by scientists and clinicians throughout the country have established that this drug has the ability to lower, without apparent toxic effects, the high blood sugar found in diabetics. It was placed on the market early in 1957.

Scientists at the Institute, and its grantees in non-Federal research centers, participated in the preliminary studies of this drug. They are now continuing their efforts to determine its long-term effects, its toxicology, and mode of action. It is important to determine whether tolbutamide possesses or in some way enhances certain of the key actions of insulin, for if not, then the lowering of blood sugar it brings about probably does not really help the diabetic make proper use of food

elements. There is still the possibility, too, that damaging effects may result from the drug's action on various organs, such as the liver, and on the hormone systems and enzymatic processes which regulate the metabolism of sugars.

Experience has shown that tolbutamide does effectively lower the blood sugar levels and urinary loss of sugar in relatively mild, recently acquired diabetes in older persons. It remains to be seen whether this evidence of control of the symptoms indicates action beneficial to the patient in the long run. At any rate, there is little reason to believe at this point that the drug is actually harmful.

Little if any response to the drug has been obtained in severely diabetic persons, and it is relatively ineffective in diabetics with the juvenile, "brittle" type of diabetes. Neither is it of any use in the treatment of keto-acidosis or in the control of diabetes during surgical stress. Insulin is usually effective in these problems.

There is great promise in the experimental use of tolbutamide as a tool in research that may lead to vital knowledge of now poorly understood metabolic processes.

Certainly it is important to learn more about the action of this drug, as well as of insulin, and research work along these lines is being intensified.

GRANTEE SCIENTISTS STUDY EFFECTS OF ORAL DIABETES DRUG

In research centers throughout the country, scientists aided by Institute grants are studying the effects and mode of action of tolbutamide (Orinase). Although much information has been obtained, some of it is contradictory. It must be reported now that neither the mode of action nor a definitive indication of the long-term effects of the drug are yet available. Some of the findings:

It has been suggested that tolbutamide may act by inhibiting the action of insulinase, an enzyme which, in turn, inactivates insulin; that it interferes with the production or action of glucagon, a hormone arising, as does insulin, in the pancreas, and which stimulates the release of glucose (blood sugar) from its storage depots in the body; that it stimulates the insulin-producing cells of the pancreas, which in the diabetic are presumably unable to produce insulin in adequate amounts.

Scientists have been trying to prove or disprove all of these theories, with varied results. Several grantees have presented evidence favoring the concept that tolbutamide acts by stimulation of the pancreatic cells, but much contradictory evidence has also been presented. Should it be found definitively that

the drug does act by stimulating the insulin-producing cells, and if long-continued stimulation by the drug does not ultimately harm them, then tolbutamide probably has a definite and valuable place in the long-term treatment of diabetes.

NEW METHOD GETS EXACT DATA ON DIABETES DRUG'S ACTION

Clinicians at the Institute have devised a new technique which provides a means for obtaining valuable information in studies to determine the mechanism of action whereby tolbutamide (Orinase) produces its effects in the body. The new oral antidiabetic drug has been prepared in a solution that can be safely and rapidly infused intravenously in a fashion similar to that used for studies of the action of insulin. The method makes it possible to obtain precise information on the action of this agent on internal organs and tissues which will be directly comparable to similar information obtained about insulin. An intravenous test dosage of tolbutamide has been determined which will give in different patients an effect in lowering the blood sugar level equivalent to that of the standard insulin intravenous test, thus providing directly comparable and precise data. Additionally, these scientists have developed a method for determining the blood levels of the drug itself, which has revealed that it is usually completely metabolized and excreted from the body within 24 hours.

Clinical studies using this new technique, supported by <u>in vitro</u> tissue work, indicate that tolbutamide interferes in some way with the output of glucose by the liver, strongly suggesting that it does not act primarily by enhancing the peripheral action of insulin produced in the patient's own pancreas. (Dr. Thomas F. Frawley, Metabolic Diseases Branch)

DBI, ANOTHER ORAL DRUG FOR DIABETES, IS TESTED

Another antidiabetic drug, more recently introduced than tolbutamide and as yet restricted to experimental use, is under investigation by research scientists, including a number of Institute grantees. Known as DBI, the drug is chemically termed phenethylformamidinyliminourea. Preliminary studies indicate that the new compound is generally effective in lowering the blood sugar level of diabetic patients --even in some who do not respond to tolbutamide, such as juvenile diabetics. In a comparatively small series of patients, one investigator found that the drug brought on distressing gastrointestinal side effects in about one-third, but that reduction in dosage decreased these symptoms.

Early reports of preliminary studies on DBI by an Institute grantee at the University of Washington (Dr. R. H. Williams) reveal several apparent variations from the effects noted with the earlier drug, tolbutamide. He found evidence that DBI inactivated insulinase, an enzyme of liver which destroys insulin. His studies also revealed that it lowered the blood sugar level in test animals even when their pancreases were removed, indicating that it was effective in the absence of insulin, an effect not shown with tolbutamide. This work also demonstrated that DBI, in the intact animal, had no effect upon the peripheral utilization of glucose and that it interfered with release of glucose from the liver. These latter two effects were also noted with tolbutamide.

Chemically, DBI is not related to tolbutamide and is not a member of the sulfonylurea family. Early results of studies of limited extent indicate that its effects, while similar to those of tolbutamide, are obtained in a different manner. Comparison of the structure of DBI with that of related compounds reveals considerable similarity to Synthalin, one of the earliest oral hypoglycemic agents, abandonded because of high toxicity.

SURGERY IN MINIATURE PRODUCES NEW DIABETES RESEARCH TOOL

By the application of the most delicate surgical techniques, employing a microscope and miniature instruments, an Institute scientist has perfected a new method for "total" (99.5%) removal of the pancreas from experimental rats. Basic research into the causes and effects of diabetes involves extensive use of experimental animals which have been made diabetic by various chemical and surgical means. These methods have produced diabetes of varying degrees of severity and have required considerable amounts of time for the diabetes to develop.

The new method provides what is essentially a new tool for diabetes research in that diabetes in these totally pancreatectomized animals occurs rapidly. Within two hours after the operation, the animals show elevated levels of blood sugar and other indications of rapidly approaching diabetic keto-acidosis, and will die within a few more hours in diabetic coma unless insulin is administered.

This rapid onset of severe diabetes is made possible by the practically total surgical removal of the pancreas, source of insulin. If even five percent of the pancreas remains, diabetes may not occur or may be greatly delayed. The perfection of this technique, involving extremely delicate surgery, provides a research tool of great value heretofore unavailable.

Using these severely diabetic rats, the scientists have been able to demonstrate the close association of disturbances in fat metabolism with the alterations of carbohydrate metabolism. Development of diabetic keto-acidosis occurred simultaneously with blood sugar elevation within two hours after the pancreas was removed. Administration of insulin corrected these evidences of altered fat metabolism at a rate similar to the reduction in blood sugar, yet other observations showed that the fat disturbance was not specifically due to lack of insulin. Inquiry is now being made into the influence on ketosis of other factors known to have various effects on diabetes, such as administration of fats, starvation, pregnancy, and growth hormone. (Drs. Robert O. Scow and Sidney S. Chernick, Laboratory of Nutrition and Endocrinology)

MUSCLE CELLS INDICATED AS SITE OF INSULIN ACTION

Precisely where and exactly how insulin works to obtain its effects is not known, but substantial support for the theory that a principal, if not the most important site of action lies in the transport of sugars across cell membranes in muscle have been provided in recent work at Vanderbilt University supported by an Institute grant. The manner in which sugar is transported across the muscle membranes is not simple diffusion, but appears to involve combination of the sugar with a molecular constituent of the cell membrane. Not only glucose, but galactose and other important sugars, it was shown, moved rapidly into muscle cells under the influence of insulin. As a result of the findings it now seems clear that there is no qualitative difference in the insulin response of heart, diaphragm, and skeletal muscle.

These extensive studies have also provided an explanation for the puzzling fact that fructose (fruit sugar), although it raises the blood sugar level, fails to overcome or relieve the dizziness, weakness and other symptoms associated with low blood sugar. The investigating scientists found that this particular sugar was unable to pass through the blood-brain barrier and enter brain cells. (Dr. C. R. Park, Vanderbilt University)

DYNAMIC NATURE OF BODY'S SUGAR STOREHOUSE REVEALED

The ideally flexible and efficient manner in which the body stores sugar (glucose) so that it is immediately available when needed for energy production has been revealed in detail for the first time by Institute scientists. Sugar is stored in the body in the form of glycogen, principally in the liver and muscles. Sometimes called "animal starch," glycogen was demonstrated by the Institute biochemists to be ideally suited for its functions as a ready storehouse of energy.

Unlike the proteins, such as insulin, glycogen molecules in a dynamic pattern of constant activity are ever-changing in size. The studies indicate that individual glucose units or residues are being added to or separated from the parent glycogen molecules in an almost never-ending process, some molecules growing while others are shrinking, so that the glycogen molecule in the living animal is never finished, never static.

This dynamic activity and constant alteration in the glycogen molecule contrasts strongly with the standardized uniformity of protein molecules. Proteins, such as hemoglobin and insulin, have specific molecular weights and are thought to be molded sequentially, molecule after identical molecule in a process which never varies. This difference in nature is what makes glycogen so well-adapted to its function, for glucose molecules can readily be deposited when available for storage and quickly mobilized for energy production when needed. Tremendous amounts of glycogen can be stored in muscle and liver without complications because of its relatively large molecular size and limited solubility. (Drs. Dewitt and Marjorie Stetten, Laboratory of Biochemistry and Metabolism)

DIABETES STUDY SHOWS EFFECTS OF UNDERFEEDING

It is known that a large proportion of persons developing diabetes after middle age are more than moderately overweight - obese, in fact; generally a sign of overeating. It is also known that comparatively mild cases of diabetes acquired in later life often can be controlled by restrictions in diet alone, without drugs. Careful diet management, too, is an important component of treatment in such patients as cannot be controlled by this means alone. Information concerning the effects of semi-starvation and acute starvation, contributing further significant information on the effects of diet, has been obtained by scientists at the State University of Iowa in work supported by an Institute research grant. Using experimental animals (rats), the investigators demonstrated that animals underfed until they lost weight and then fasted for 24 hours were able to clear an administered dose of glucose (blood sugar) from their bloodstreams more rapidly than did well-fed animals. Along with other related findings, this would seem to indicate that the underfed animals develop some inherent mechanism which enhances their ability to utilize glucose. These studies may lead to a better understanding of the effects of underfeeding in the control of diabetes. (Drs. N. S. Halmi and B. N. Spirtos, State University of Iowa, Iowa City)

DIABETIC NEURITIS NOT CAUSED BY VITAMIN LACK

In a study designed to clarify conflicting notions concerning vitamin deficiencies as causative factors in the numbness, tingling, and pain associated with diabetic neuritis, Institute scientists have shown that the lack of B vitamins does not cause this type of complication in diabetics. A correlative conclusion is that the administration of B vitamins to a diabetic with such complications will be ineffective.

A precisely controlled, carefully planned study was made of vitamin tolerance and excretion in 21 patients, seven being normal subjects, seven diabetics with no complications, and seven diabetics with neuropathy. Significant differences among the three groups were minor, and indicated no clear relationship between patterns of vitamin utilization and the presence of degenerative nerve disease.

Since the degenerative complications of diabetes make their appearance over the span of a number of years, the comparatively brief period covered by this study must be considered, but the elaborate design and careful controls employed permit the definitive statement that no basis was found for believing that an abnormal B vitamin metabolism is a causative factor in the development of the degenerative lesions of diabetes. Aside from the importance of its findings relative to the role of the B vitamins in diabetes, this study serves to point up again the fact that diabetes is a complex disorder, not subject to easy analysis and understanding, even in small segments. (Dr. James B. Field, Metabolic Diseases Branch)

RHEUMATIC DISEASES

Although in recent years new and better antirheumatic drugs have been developed, and newer ones now are being tested, the treatment of the rheumatic diseases is still only palliative. Symptoms can be suppressed, patients made more comfortable, and crippling can sometimes be prevented, but still there is no cure and the cause remains unknown.

Those leading and participating in the research attack upon these crippling diseases have turned their attention largely to fundamentals, directing more effort toward discovery of the underlying factors. Such research seems vital, for until we know more of their nature it is unlikely that the means for prevention and cure will be developed.

Advances on several fronts have been recorded during the past year as chemists, pharmacologists, epidemiologists, clinicians, physicists, and biochemists have concentrated on basic problems associated with arthritis and other rheumatic diseases. These disorders, including rheumatoid arthritis, osteoarthritis, fibrositis, bursitis, gout, lupus erythematosus, scleroderma, and dermatomyositis, among others, afflict more than 10 million people in this country.

SIMPLE, RAPID TEST DETECTS RHEUMATOID ARTHRITIS

A new diagnostic test for rheumatoid arthritis which is so simple and rapid that it can be performed in a routine clinical laboratory in about 20 minutes was developed during the past year in the Institute's clinical laboratories. Known as the Bentonite Flocculation Test (BFT), it has a number of advantages over older tests. As accurate as the best of these, it has the added practical advantages of being simple and rapid, producing results in minutes rather than in hours or days, and requiring only simple, easily available materials and equipment. The simplicity of the new test makes it possible for the average medical technician or physician's assistant to conduct 100 or more such tests per day. For this reason it may become the first of such diagnostic tests to be widely used by physicians.

The procedure, developed in a collaborative project by scientists of the National Institute of Arthritis and Metabolic Diseases and the National Institute of Allergy and Infectious Diseases, employs as its key element a type of colloidal clay known as Bentonite, which is mixed with normal human gamma globulin. A drop of blood serum from the person being tested is added to a drop of the

Bentonite-gamma globulin mixture on a slide. If the test is positive, the Bentonite particles will clump (flocculate) within a few minutes. The tiny clump is detected under a microscope. There is a sharp delineation between normal and abnormal values.

Bentonite, a colloidal form of clay, is stable, inert, and easily available commercially. It is used in industry for such varied purposes as clarifying wine and beer, stopping water leaks in pipes, and as a binder in making briquettes from charcoal.

The tests, in trials conducted at Bethesda, were positive in 85 percent of 82 cases of verified rheumatoid arthritis. In 227 controls, on the other hand, it was negative in 98 percent. These findings have been confirmed by similar experience in other laboratories now using the test. Thus, in its early stages of development, the test appears to be able to detect accurately almost nine out of 10 cases, and to yield "false" positives in less than two out of 100 cases.

As in other serological tests for rheumatoid arthritis, the BFT is based upon the well-established fact that persons with active rheumatoid arthritis have in their blood serum a "rheumatoid factor" which has the capacity to cause sensitized particles to clump, or flocculate. Particles previously used have included red blood cells from man, sheep, and other animals, collodion, and latex. (Drs. Joseph J. Bunim, Clinical Investigations, NIAMD; Jules Freund and John Bozicevich, Laboratory of Immunology, NIAID)

RHEUMATOID ARTHRITIS SHOWS FAMILIAL TRAITS

Reports of investigators in the United States and England have recently indicated that there is mounting evidence here and abroad that rheumatoid arthritis has a genetic or familial trait, that it tends to run in families.

On the basis of reactions to the sheep cell agglutination diagnostic test for rheumatoid arthritis, it has been shown that members of the families of arthritics, even though they have no clinical symptoms of the disease themselves, reflect a higher rate of positive reactions than do normal controls.

An Institute grantee at New York University has reported that individuals without symptoms, but with a family relationship to an arthritic, had a rate of 18 percent positive tests, while normal control subjects similarly tested showed a positive response of only 2.5 percent. (Dr. Morris Ziff, New York University, New York)

Meanwhile, in England, Dr. J. S. Lawrence, in a similar survey with the sheep cell agglutination test, found an even higher percentage of positives among relatives of arthritics and noted, additionally, that in those family groups, persons over the age of 65 who had no clinical symptoms of rheumatoid arthritis showed a rate of positive tests that went up to 30 percent.

OSTEOARTHRITIS SUSCEPTIBILITY MAY BE INHERITED, STUDY SHOWS

A detailed study of a large group of intermarried families having a high incidence of osteoarthritis is being conducted by Institute clinicians in an effort to learn more concerning the nature of this widely prevalent disorder. Very little, actually, is known about the cause, predisposing factors, or even the course of osteoarthritis, which is attributed largely to wear and tear upon the structure of, particularly, the weight-bearing joints.

The study, still incomplete, has contributed information which confirms findings in experimental animals to the effect that (1) susceptibility to osteoarthritis is genetically transmitted, and (2) in those with inherited susceptibility, obesity contributes significantly to the occurrence of the disorder.

The family group under study is composed of approximately 400 members, fairly stable, and residing in a small geographic area. There is evidence of some degree of in-breeding. Early information concerning the family indicated a high incidence of "back trouble," while a preliminary survey revealed severe generalized osteoarthritis in a number of individuals of short stature who were considerably overweight, or obese.

To date, although none of the persons studied under this project have been admitted to the Clinical Center as in-patients, 52 individuals have been examined and appropriate laboratory work accomplished. They have been divided into three groups: (1) under 20 years of age, (2) between 20 and 39, and (3) between 40 and 79. None in group (1) had osteoarthritis; six of the 16 in group (2) had osteoarthritis of the spine, but no peripheral joint involvement. All 12 patients in group (3) had osteoarthritis of the spine, and 10 of the 12 had involvement of the peripheral joints as well. (Dr. Roger L. Black, Arthritis and Rheumatism Branch)

ARTHRITIS DRUGS EVALUATED IN CLINICAL TEST PROGRAM

During the past year Institute clinicians have conducted an active evaluation program involving a number of new anti-inflammatory compounds, along with their continuing long-term studies of the effects of prednisone and prednisolone, now well-established in medical practice in the treatment of rheumatoid arthritis.

Among the new drugs tested were triamcinolone (a modification of prednisone) and several other new steroids, most of them variations of prednisone and prednisolone, synthesized by pharmaceutical chemists in their continuing attempts to produce drugs of improved potency and minimal side effects. Also tested were an antimalarial compound known as chloroquine and two derivatives of another type of antirheumatic drug, phenylbutazone.

Prednisone and prednisolone now have been evaluated in 59 Clinical Center patients with rheumatoid arthritis, each receiving a minimum maintenance dosage. Of these, 14 have been followed regularly in a long-term study. Seven of these 14 have attained full functional capacity, two others are only slightly handicapped, and four are moderately handicapped. One is confined to a bed-chair existence. Although these results indicate that these drugs are effective antirheumatics, serious side effects have been a problem in a number of patients as a result of long-term use.

Results in comparatively short-term, limited tests of other drugs indicate that triamcinolone has about the same potency as prednisone and apparently few advantages, although on the short-term test no serious side effects developed. A group of four other synthetic steroid compounds tested showed unimpressive antirheumatic properties, and it was concluded that they would be of little value in the treatment of rheumatoid arthritis. Another synthetic steroid compound, however, checked out well in nine patients, having an antirheumatic potency (on a weight basis) equal to that of prednisone. Two patients developed undesirable side effects.

One of the phenylbutazone derivatives, known as G-27202, provided moderate objective and subjective improvements in patients, but the other (G-33) produced no effect. Results with chloroquine are not at this time of such nature as to permit evaluation, a much longer term of trial being indicated. (Dr. Joseph J. Bunim, Arthritis and Rheumatism Branch)

RHEUMATOID FACTOR ISOLATED, DESCRIBED; ACTIVITY MEASURED

A substance known as the "rheumatoid factor" found in the blood of persons with active rheumatoid arthritis has made possible the development of diagnostic tests for the disease, discussed earlier in this report. It has the capacity to agglutinate various sensitized particles with which it comes in contact, causing them to clump in a characteristic fashion.

Because this factor may provide important information as to the cause of rheumatoid arthritis, which is not now known, and because it may also provide additional information as to the basic nature of the disease itself, which will prove valuable in developing possible means of preventing it or better methods of treatment, considerable research of a fundamental nature involving it is under

way, not only at the Institute but in other laboratories throughout the country.

Scientists at the Institute, in the course of attempts to isolate and characterize the rheumatoid factor, have developed a new and precise method of measuring its activity which has a margin of error of only 5 percent. Previous methods varied in error from 100 to 200 percent. (Dr. R. R. Williams, Laboratory of Physical Biology)

At the Medical College of Virginia, a substance which interferes with the agglutination reactions of the rheumatoid factor was identified by an NIAMD grantee as a filterable, extremely small type of gamma globulin. It exists in the serum in great excess but in loose combination with the factor itself. (Dr. John Vaughan, Medical College of Virginia)

In work reported at the Interim Scientific Session of the American Rheumatism Association held recently at the Clinical Center in Bethesda, Drs. Edward C. Franklin and Henry G. Kunkel of the Rockefeller Institute, New York, succeeded in isolating the rheumatoid factor in 95 percent purity, determining that it is a gamma globulin protein.

STUDY CLARIFIES ASPIRIN'S ROLE AS AN ANTIRHEUMATIC

Because many patients with rheumatic disease have responded to the administration of aspirin in a manner similar to the response obtained when steroid hormones such as cortisone and prednisone are used, it has been suggested that aspirin might act by stimulating the adrenal gland, which secretes steroid hormones.

Methods largely developed by clinical investigators at the Institute have recently made it possible to measure precisely the activity of the adrenal gland, hour by hour. Employing these methods in a clinical study, Institute scientists now have demonstrated that aspirin acts independently of the adrenal gland to bring about its antirheumatic action. The effect aspirin has is not, on the basis of these findings, dependent in any way upon adrenocortical steroids. Administration of salicylates did not, during long-term administration, increase the rate of synthesis or secretion of these steroids in either normal or rheumatoid patients. In the course of the study, one patient who was known to be responsive to a specific amount of aspirin daily responded in the same manner to the same amount of aspirin even when his adrenocortical function was entirely suppressed. (Dr. R. E. Peterson, Arthritis and Rheumatism Branch)

PURINE METABOLISM RESEARCH YIELDS INFORMATION ON GOUT

Research involving the metabolism of purines is of vital importance in the search for improvement in our understanding of gout, for the purines, including xanthine and uric acid, play exceedingly important roles in that disease. The purines are substances which are synthesized in the body. They are also present in various items in the diet, particularly in meats such as liver and kidney. One of the clinical features of gout is an abnormal high level of uric acid, a purine, in the blood. A characteristic feature in chronic tophaceous gout is the deposition of urate salts in the joints, arising from the excessive uric acid in the gouty person's system.

Findings by an Institute grantee, recently reported, furnish a clue to the obscure relationship between the acute arthritis attacks of gouty patients and the abnormal purine metabolism in this disease. He has detected a purine metabolite, or intermediate breakdown product, in the urine of gouty patients, and has noted that it increases in amount during attacks of gouty arthritis. This discovery constitutes the first indication of a specific link between the clinical and metabolic features of this disorder -- a breakthrough in the long scientific effort to establish such a connection. (Dr. B. Weissmann and associates, Mt. Sinai Hospital, N. Y.)

In other work in purine metabolism an Institute investigator has found evidence of a regulatory mechanism controlling the production of uric acid in the body. This finding suggests the possibility that a faulty control system is responsible for the overproduction of uric acid in certain gouty patients. It is entirely possible that this metabolic defect is inherited. (Dr. J. E. Seegmiller, Arthritis and Rheumatism Branch)

METABOLIC DEFECT IN GOUT FINALLY DETERMINED

Although it has been known for many years that patients with gout have excessively high levels of uric acid in their blood, there has been a continuing controversy as to why this is so. It could occur because of excessive production, insufficient destruction, or inability to excrete proper quantities of the material.

Using radioactive tracer techniques much more specific than those previously used in studies of gout, an NIAMD scientist has now conclusively demonstrated that overproduction of uric acid is the effective metabolic defect in primary gout. This definitive work would appear to explain the immediate cause of the presence of uric acid in excessive amounts in the gouty person's blood, and points the way to further research which will ultimately reveal the intimate details of the metabolic error which causes this overproduction. (Dr. James B. Wyngaarden, Metabolic Diseases Branch)

COLCHICINE BY INJECTION FOUND MORE EFFECTIVE THAN BY MOUTH

Colchicine administered orally has for many hundreds of years been used in the treatment of gout and is still perhaps the most effective agent now in common use to suppress the exquisitely painful symptoms of gouty arthritis. However, most persons using the drug experience extremely distressing gastrointestinal effects brought on by it. This limited the drug's usefulness in some cases, although it must be admitted that the extreme pain suffered by the patient with acute gouty arthritis usually left little choice.

A systematic evaluation of colchicine injected into the veins of a test series of 40 patients by Institute clinicians revealed that the drug administered in this manner caused no lasting or serious side effects, and that the intravenous injection method produced relief of the acute gouty attack more promptly. Gastrointestinal effects were minimized. The evaluation program was undertaken following the recent introduction of a newly available intravenous preparation of colchicine which gave promise of being an improvement over former preparations which had not been entirely satisfactory.

This finding will be welcomed particularly by patients whose gastrointestinal reactions to orally administered colchicine have been severe, even though intravenous injection is not so easily accomplished as is oral administration. (Drs. J. E. Seegmiller, K. Lemone Yielding, and Leonard Laster, Arthritis and Rheumatism Branch)

MOLECULAR DISEASES

Important advances in biochemical knowledge and techniques during the past four years have resulted in the development of an entirely new concept of "molecular diseases." Certain hereditary metabolic disorders which have been found to be due to the partial or total lack of a specific enzyme are assigned to this group of diseases.

The term "molecular" is applied to these diseases because of the fact that the enzymes which are missing or defective have definite reproducible protein molecular structure. As with all proteins, the production of these enzymes is under genetic control, produced by cells which generate identical molecule after molecule.

Thus, in the molecular diseases, due to an inherited defect, the afflicted person's system is not able to produce an effective enzyme which is necessary to set a specific metabolic process into operation. In the absence or ineffective action of this process, death in infancy or early serious disease results.

As we dig deeper and learn more about the basic life processes, it is entirely possible that more and more metabolic diseases will be revealed as "molecular" in nature.

Two years ago the discovery by Institute scientists of the cause of galactose diabetes (galactosemia) was reported. This discovery revealed the molecular nature of this metabolic disorder. Important additional findings have been made concerning it during the past year, and two more diseases, alcaptonuria and congenital non-hemolytic jaundice, have been added to the list.

MORE FACTS LEARNED ABOUT GALACTOSEMIA

Discovery of the cause of galactosemia by Institute scientists in 1955 led to the development of a relatively simple diagnostic test for this molecular disease which, although thought to be comparatively rare, has caused the death of many infants whose condition could not be promptly diagnosed. In this disease the absence of a single enzyme makes it impossible for the afflicted infant to digest properly one of the sugars in milk, bringing about a toxic condition which, if not promptly and accurately diagnosed, leads to progressively more serious complications involving jaundice, mental retardation, and early death. Once diagnosed, removal of milk from the diet terminates the development of the condition.

Further work has unearthed an explanation for the fact that patients with galactosemia, while still lacking the essential enzyme, show an increased ability to tolerate milk as they advance in age. A second pathway for the breakdown of milk sugar has been found. The enzyme involved is very feeble in early life but gradually increases in activity, which explains the clinical observation that galactosemic patients build an ability to digest milk as they grow older. Thus, infants with galactosemia whose conditions were diagnosed early, and who have developed normally on a diet free of milk and milk products, can look forward with some degree of assurance that eventually they may be able to handle modest amounts of milk and milk-containing foods when they are older.

CAUSE OF ANOTHER MOLECULAR DISEASE FOUND BY SCIENTISTS

A significant medical research finding by a team of scientist-physicians at the Institute has proved, with precise biochemical evidence, that the comparatively rare hereditary disease, alcaptonuria, is caused by the absence of a single protein enzyme, identified as homogentisic acid oxidase. Although the general nature of the disease was known, and the cause was suspected, its exact nature never had been scientifically demonstrated.

Particular importance is attached to this finding because of the fact that the basic disorder is often accompanied by a form of arthritis similar to rheumatoid spondylitis, affecting the spine. This demonstrated relationship may provide guidance in the search for the cause of other types of arthritis.

Although persons with this disease are born with the basic defect in their metabolism, the disorder, during the first two decades of life, is little more than socially disturbing, since it is distinguished only by the presence in the urine and perspiration of a substance which turns brown or brownish-black upon exposure to air. The soiled diapers of afflicted infants display the characteristic discoloration, while adults find that their perspiration stains articles of clothing. Serious complications, however, including arthritis and arteriosclerosis, often develop as middle age approaches.

The researchers responsible for this finding, Drs. LaDu, Seegmiller, and Laster, of the Arthritis and Rheumatism Branch, are now following up their discovery by seeking information as to the precise manner in which alcaptonuria is related to the resultant arthritis in middle age. The fact that some patients with alcaptonuria do not develop joint symptoms may lead to a method of preventing its occurrences in all cases and may result in information which will lead to findings related to the cause or causes of other types of arthritis not associated with this particular disorder.

CAUSE OF CONGENITAL TYPE OF JAUNDICE FOUND BY SCIENTIST

A type of jaundice which afflicts children, in particular, has been characterized by an Institute scientist as a "molecular" disease which is caused by the congenital lack of the enzyme necessary to convert bilirubin, one of the constituents of bile, into the form in which it can be excreted. Known as congenital non-hemolytic jaundice, this disorder, which gets its name from the fact that the red blood cells are not broken down, is apparently hereditary. As the bilirubin, a bile pigment, cannot be excreted until it has been conjugated with glucuronide, and as this can be done only in the presence of the missing enzyme, the bilirubin piles up in the blood, bringing on the disorder.

A well-planned combination of laboratory, animal, and clinical studies led Dr. Rudi Schmid of the Metabolic Diseases Branch to his conclusions. Patients with this type of jaundice can be clearly distinguished from those with jaundice due to obstructions, who also cannot excrete bile into the intestines, by the fact that the blood and urine of the latter both contain conjugated bilirubin.

BASIC RESEARCH: METABOLISM, NUTRITION

Hormones, enzymes, and vitamins play leading roles in the intricate metabolic processes of the body. They are the key compounds that initiate, mediate, and control the complex system by which the metabolic fuels--food, air, and water--are converted into growth and energy, thus maintaining the structure of the body and the functions of life.

A missing enzyme, an inadequate hormone, a vitamin produced in insufficient quantity--any of these can cause the disruption of a metabolic process important to continued life and health. Detailed knowledge of the manner in which these compounds operate--how they affect and interact with each other to achieve their effects--is important. Such knowledge has recently been furthered by Institute scientists and grantees.

BASIC BIOPHYSICAL STUDIES PROVIDE SPACE TRAVEL DATA

For several years physical biologists of the NIAMD have been concerned with problems affecting life and health at high altitudes. These studies have produced findings applicable in the suddenly important era of rockets and satellites. One series of experiments, involving problems of human energy expenditure in underwater work and at simulated high altitudes, has yielded information useful in calculating oxygen needs in confined environments. It has been found that removal of expired carbon dioxide and water vapor do not raise difficult problems, but that handling the heat generated from metabolism, from solar radiation, and from friction on the outer surfaces of such vessels as space ships and satellites requires exceptional engineering skill. These very difficult thermal problems will become progressively less difficult as it becomes possible to carry large payloads, more equipment, in space-traveling vehicles. (Dr. Heinz Specht, Laboratory of Physical Biology)

Bombardment by cosmic rays is a serious consideration in space travel. The problems posed by cosmic radiation have been under study by Institute scientists for a number of years. On the surface of the earth we are protected from the harmful effects of cosmic rays by the earth's atmosphere, which is as effective as a wall of lead three feet thick. Nevertheless, a significant number of these harmful rays do penetrate the atmospheric screen. Near the top of the atmosphere, an Institute scientist has found, each cubic centimeter of tissue receives a heavy dose of cosmic radiation, generally composed

of the nucleii of such heavy elements as iron and nickel. Tissues particularly sensitive to this radiation are the lens and retina of the eye, nerve cells, dermal papillae, and hair follicles. Tracks and recordings of cosmic rays have been captured in specially designed thick photographic emulsion packets sent aloft in high-altitude balloons in collaboration with the Air Force. (Dr. Herman Yagoda, Laboratory of Physical Biology)

In other related biophysical studies Institute scientists have been studying the influence of high altitude on susceptibility to disease. Test animals have shown a tendency to develop vegetations on heart valves and thickening of valvular tissue after long exposure to altitudes of only 25,000 feet. These effects are attributed to lowered pressure and oxygen tension, suggesting the importance of precise pressure and oxygen control in sealed cabin environments. (Dr. Paul D. Altland, Laboratory of Physical Biology)

That obesity and high fat diets are dangerous at high altitudes is also indicated by these studies. Obese rats and rats on high fat diets showed extreme sensitivity and a tendency to sudden death upon acute exposure to high altitudes. (Altland, ibid., and Dr. Olaf Mickelsen, Laboratory of Nutrition and Endocrinology)

BONES OF HUMAN SKELETON ARE DYNAMIC, NOT INERT

Once considered to be altogether inert, the human skeleton has recently been shown to be subject to continual turnover, just as are other body tissues, such as skin. Bone, it is now known, is continually wearing out and being replaced in the normal human skeleton. This turnover of bone and the active formation of new bone in the normal adult skeleton has now been demonstrated by Institute investigators to be much more extensive than previously supposed. Using a new technique for handling radioactive calcium data, devised in NIAMD laboratories, these scientists have demonstrated that the predominant mechanism, in both the adult and growing skeleton, is actual physical destruction of structural bone units and their replacement by new bone formation. Normal adult bone formation, they found, results in the deposition of approximately 600 milligrams of calcium daily, an amount approximating the average daily dietary intake of the mineral.

An unusual and interesting finding in this connection was in relation to patients with osteoporosis, a disease in which the bones become gradually thinner and more brittle and which occurs primarily in the aged and in post-menopausal women. Consistently in five patients the bone formation rates were found to be normal, an observation contrary to the generally accepted idea that this disorder is due to diminished bone formation. The study indicates that osteoporosis may actually be due to increased bone destruction and indicate the need for additional intake of dietary calcium - as in milk and milk products. (Drs. Robert Heaney and G. Donald Whedon, Metabolic Diseases Branch)

WOUND HEALING SPEEDED BY POWDERED CARTILAGE

Local application of powdered cartilage to wounds at the time of surgical repair has been found to speed the healing process significantly and to increase the tensile strength of sutured abdominal wounds by 20 percent. Physicians at Columbia University, in work supported by an Institute research grant, also found that powdered bone, talcum powder, gelatin and methionine had no influence on the healing of experimental surgical wounds.

The specific fraction of cartilage responsible for the effects obtained has not as yet been isolated or characterized, but these initial observations are being followed by more detailed studies into the sequential histological and biochemical changes in healing wound tissue.

The cartilage powder used in these experiments was commercially prepared from bovine cartilage. Cartilage is largely composed of connective tissue, detailed studies of which are important in rheumatic disease research. Rheumatic diseases, including rheumatoid arthritis, attack, inflame, and sometimes destroy the connective tissue throughout the body. (Dr. John F. Pruden and associates, Columbia University, N.Y., N.Y.)

NEW DRUG MAY WORK WHERE MORPHINE FAILS

The separation of pain-killing (analgesic) action from addiction liability in substances having potency as great or greater than morphine has for many years been an unattainable goal for chemists. Morphine in optimal doses will adequately control only 80 percent of severe clinical pain. Because of this there is a definite need for a drug which will affect areas not reached by morphine.

Chemists at the National Institute of Arthritis and Metabolic Diseases, searching for new, more potent, and less addicting drugs, have developed a promising one known simply as NIH 7519 which they synthesized, simply, from coal tar derivatives. This new drug is 10 to 12 times as potent as morphine, and though presumably just as addicting, it appears to have a wide range of safety. As of the present, it constitutes a most promising candidate for use in the relief of severe clinical pain not controllable with morphine at safe dosage levels. Additional testing is required before the drug's practical potential can be determined. (Dr. Everette L. May, Laboratory of Chemistry)

NIAMD CHEMISTS DEVELOP PAIN KILLER SAFER THAN CODEINE — A new drug prepared by chemists at the National Institute of Arthritis and Metabolic Diseases - B-Methadol (NIH 4543) — in tests on experimental animals has proved to be somewhat more effective in killing pain than either codeine or Demerol, with no addiction liability. Addiction tests have been carried out in monkeys at the University of Michigan and in man at the Public Health Service Hospital, Lexington, Kentucky. In both cases the addiction tests were negative. Plans are under way for clinical trials of this easily synthesized drug which shows promise as a competitor with both Demerol and codeine, since it appears more effective, yet is without the addiction hazards these two commonly used drugs present. (Drs. Everette L. May, Nathan Eddy and Erich Mosettig, Laboratory of Chemistry)

BETTER NUTRITION BENEFITS POST-OPERATIVE PATIENTS — The value to surgical patients of measures to provide adequate nutrition during the immediate post-operative period has been demonstrated in metabolic balance studies carried out by Institute grantees at Western Reserve University. They found that preservation of body protein stores and maintenance of body weight could be accomplished by easily administered and well-tolerated intravenous protein and calorie feedings.

The NIAMD supported investigators, working with normal subjects as well as with patients requiring various minor and major surgical operations, demonstrated that nitrogen losses formerly thought to be the result of operative injury or stress are in fact almost entirely the result of poor nutritional intake. Normal subjects, deprived of protein and calories to the same

extent and for the same three-to-five day period as post-operative patients managed in the traditional semi-starvation fashion, were found to have nitrogen and body weight losses of virtually the same degree as the patients.

Using a series of patients who underwent a gastric resection as a uniform type of surgical trauma, the surgeons also found that adequate calories and nitrogen in the form of protein hydrolysates and sugar solutions maintained metabolic balance. Sugar solutions alone were almost entirely ineffective. It was found that 80 grams of protein per day held nitrogen losses to a minimum, while 100 to 120 grams were completely protective. The investigators observed that patients supported by increased caloric and nitrogen intake seemed better able to withstand post-operative complications and recovered more rapidly and vigorously than did patients managed under the formerly used semi-starvation regimen. (Dr. William D. Holden and associates, Western Reserve University, Cleveland)

USE OF SEX HORMONES IN FOOD PRODUCTION REVIEWED

An important development in the food production industry in recent years has been the administration of estrogenic compounds to beef cattle and poultry to improve growth and reduce feed requirements. The practice of "hormonizing" beef cattle started less than three years ago, but already is firmly established. It is estimated that approximately two-thirds of all cattle in feed lots in the United States, in addition to many millions of chickens and turkeys, are being treated with these estrogen-active compounds.

Institute nutritionists have followed this development with great interest, as they are familiar with some of the problems involved, having conducted work in this area. The public health aspects of this development recently were reviewed in a survey of current scientific literature on the subject by an Institute scientist.

In making a determination as to the possible dangers to human beings involved in eating meat from estrogen-treated livestock, the most important measurement is the amount of estrogen residues in such tissues. In nine reports reviewed, all agreed that only approximately one microgram or less of estrogenic activity can be found per 100 grams of tissue by the most sensitive assay methods. Thus, unless an individual consumes excessive quantities of meat, he can expect to ingest barely more than one microgram (one-thousandth of a milligram) per day. This amount of estrogenic hormone is no greater than is

contained in an ordinary diet of milk, leafy vegetables, and meat from untreated animals.

The Institute scientist's conclusion is that there is no danger to the public health involved in the practice insofar as it might stem from the eating of meat from animals or poultry treated with estrogens. However, he did conclude that there might be some potential danger involved among persons employed in plants manufacturing the compounds.

Reasons why the use of estrogen compounds in the cattle-raising and poultry-raising industries has spread so rapidly can be gleaned from the fact that the demonstrated effects include increases in weight of as much as 20 percent (average: 12 percent), frequently improved carcass quality, and an average saving of about 10 percent on feed. Preparation of the feed containing estrogen compounds is closely supervised by the Government. (Dr. George Briggs, Laboratory of Nutrition and Endocrinology)

STUDY SHOWS 10% OF SCHOOL CHILDREN TO BE OVERWEIGHT

The most difficult type of obesity to control is the well-established, long-standing kind. A study of the incidence of obesity among school children in the Greater Boston area of Massachusetts may point the way toward means of prevention of the long-standing type by correction of the tendency in childhood.

Surveying and interpreting school records in two Boston communities, investigators at Harvard University, in work partially supported by an Institute grant, using the Wetzel Grid as a basis for the definition of overweight, found that more than 10 percent of the 6,346 students in 10 public schools were overweight. Height-weight data from current physical examinations were obtained from school authorities. The schools selected for study comprised well-to-do as well as poorer sections of the communities. From the point of view of development two types of overweight were found to be most common: "persistent obesity" present throughout the school record, and "late obesity," present only during the latter half of the school record. The persistent type occurred in a third of the overweight girls and almost half of the overweight boys, while the late, or more recently acquired type in older children occurred in more than a third of the overweight boys and in 12 percent of the girls. It was noted that the onset of overweight took place largely in the winter months, which may be

taken as emphasizing the importance of inactivity in the causation of many cases of obesity. (Dr. Mary Louise Johnson and associates, Harvard School of Public Health, Boston, Massachusetts)

ANTIRHEUMATIC DRUGS AISO AID POISON IVY VICTIMS

Dermatologists at the University of Cincinnati have found that prednisone and prednisolone, widely used arthritis drugs, are effective in the treatment of severe skin reactions caused by poison ivy and poison oak. In work partially supported by an Institute grant, the investigators note that two important clinical factors must be considered prior to use of the potent steroid drugs, in addition to usual precautions: (1) the severity (mild, moderate, severe), and (2) the time relationship of the institution of therapy and the onset of the dermatitis. Adequate oral administration of prednisone and prednisolone can aid the dermatitis from poison ivy or oak, they found, and unless contraindicated, should be employed in severe cases, and may, with benefit, be used in moderate cases. Creams and ointments containing the steroids, they found, were of no value except in very mild cases.

The scientists point out that ivy and oak poisoning can be serious, making patients very ill and miserable, requiring extensive skin nursing care. Even a mild affliction can be irritated and become severe. Secondary infection can develop. And though uncommon, chronic complications such as a persistent localized neurodermatitis from repeated scratchings may develop, or in a patient with latent or active psoriasis, some or even all of the lesions may become psoriatic. (Drs. L. Goldman and R. Preston, University of Cincinnati)

AMOUNT OF WATER IN DIET CONTROLS TOTAL FOOD INTAKE

Water intake has a decisive effect on food intake, scientists at the University of California have found in studies supported by the National Institute of Arthritis and Metabolic Diseases. Experimental animals (rats) deprived of water with meals ate less than rats fed with water, yet the gastric contents of all animals fed with and without water was approximately 49 percent water. This indicates close regulation of water in gastric contents. When fed without water the animals regulate their food intake to match the amount of water they can mobilize from their own tissues, thereby maintaining the proper water-food ratio.

Withholding water during meals does not appear to interfere with digestion, the scientists found, but it definitely does decrease the appetite and effects a reduction of food intake. (Dr. Samuel Lepkovsky and associates, University of California, Berkeley)

TRACE ELEMENT, SELENIUM, PREVENTS LIVER NECROSIS

An odor, quite similar to that of garlic, detected by a scientist as he was checking some highly concentrated preparations, led to the discovery that the trace element, selenium, was the essential active ingredient in the dietary substance known as Factor 3, which protects experimental animals against fatal deficiency disorders. The amino acid, cystine, and vitamin E have been known since 1944 to be protective against necrotic liver disease in animals, and in 1951 Dr. Klaus Schwarz of the Institute's Laboratory of Nutrition and Endocrinology discovered a third factor which would also accomplish this. Factor 3 has also been found to prevent necrotic degeneration of liver, heart, kidney, and muscle in mice and an exudative diathesis in chicks, fatal diseases which result from a multiple deficiency in the diet of all three factors--vitamin E, cystine, and Factor 3. The selenium of Factor 3 is much more potent than either cystine or vitamin E. Only four parts to a million in the diet are effective in preventing necrotic liver degeneration in experimental animals.

Recognition by Dr. Schwarz and associates of selenium as the active element in Factor 3 represents a major step forward in the study of experimental nutritional liver disease, bringing closer the eventual understanding of the true relationship between experimental liver disease and human dietary liver injury. It should be noted that, in humans, liver necrosis of this type has not been recognized, and the requirement in human nutrition for either Factor 3 or selenium has not been established.

ADVANTAGES OF FRESH BONE TRANSPLANTS REAFFIRMED

With the introduction of the bone-bank in recent years, there has been a revival of the old controversy as to which of several types of bone transplants is superior. A majority of informed experts apparently believe that fresh bone transplants, taken from the patient's own body are better than other types, but many hold the opinion that the advantages of fresh bone transplants are not of such significance as to outweigh the

advantages to the patient of banked bone which is readily available without undue loss of time. It has been claimed by some that although banked bone is somewhat more slowly repaired in the early stages, it eventually is as good.

An attempt to clarify the problem has been made by investigators at the State University of Iowa, partially supported by an Institute grant. A series of tests on 100 rabbits with fractured bones demonstrated that fresh bone transplants taken from the injured animal's own body were better tolerated, healed more rapidly by bony union, and were more rapidly replaced by new, living bone. The investigator states unequivocally that fresh bone transplants taken from the injured animal's own body "are superior to any other type of bone transplant," and concludes that the repair capacity of such transplants, as measured by graft fracture healing, exceeds that of fresh, freeze-dried, or merthiolate grafts from other sources. (Dr. Michael Bonfiglio, State University of Iowa, Iowa City)

HIGHLIGHTS

OF

DENTAL RESEARCH

1957

Items of Interest on Program Developments and Research Studies Conducted and Supported by the National Institute of Dental Research

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One of the major deterrents in the past to the conduct of research in the dental field has been the scarcity of graduate training facilities oriented toward dental research. Consequently the inauguration of graduate research training programs in fifteen dental schools during the past twelve months is regarded as an achievement of primary importance to the future of dental research in the United States. The establishment of the training programs has been made possible through Public Health Service grant funds. The programs are specifically designed to train graduate students in the basic sciences related to the different clinical specialties of dentistry. The trainees will become the teachers and clinical research workers of the future. These training programs, coupled with an increased number of fellowship awards and individual research project grants, represent a decisive forward step in the development of an adequate, overall, dental research program for our Nation.

That there is now some research interest and activity in almost all dental schools throughout the country is evident from applications for research projects, fellowships, and training grants. If sustained, this interest cannot fail to influence the quality of dental education and to attract superior dental students to careers in research and teaching.

In addition to the increased research activity at dental schools, the expanded grants program has encouraged dental research at other institutions. Research workers in non-dental basic science departments have now begun to orient some of their efforts toward dental problems. It has also been possible to award a limited number of grants to research teams in hospitals in order to include oral aspects in research studies on general systemic disease.

Prepared, January 1958

Certainly to a larger degree than in any of the other branches of the life sciences the progress of dental research has depended upon Federal funds. Research funds from other sources have been very limited by comparison. This expanding program mentioned above, however, will not only broaden the scientific base but also increase public awareness of the aims and objectives of dental research.

Keeping pace with these developments in the country-wide programs is work done in the National Institutes of Health laboratories at Bethesda, Maryland by the scientific staff of the National Institute of Dental Research. Here greatest emphasis has been placed on basic research and this emphasis has been partially balanced by limited studies of clinical problems. Some highlights of the advances both at Bethesda and by grantees throughout the country are mentioned in the pages that follow.

FLUORIDES AND DENTAL HEALTH

AUTOPSY STUDIES Fluorides in drinking water, even at greater concentrations (2.6 parts fluoride per million) than those recommended (1 part per million), have not caused disease or malfunction in vital organs of long-term residents of Colorado Springs, Colorado. Evidence for this comes from a study by Dr. E. F. Geever, NIDR consultant, of a very extensive series of autopsies. The present study, based on this material, was designed to evaluate any possible relationship between fluoride ingestion and disease. Comparative statistical analyses of the pathologic findings revealed no significant differences which could be related to long-term residence in this environment.

In another study by Dr. Geever and his associates, additional evidence has been accumulated that fluoridated drinking water up to 4.0 ppm has no toxic effect on microscopic bone structure in man. This work is based on approximately 100 bone specimens (iliac crests, ribs and vertebrae) from 37 patients autopsied in communities with fluoride levels ranging from 1.0 to 4.0 ppm. The patients had resided in these communities for at least 10 years. Comparative microscopic study with a similar series of bone specimens obtained from patients who had lived for corresponding periods in areas where a non-fluoride drinking water is in use showed no significant differences which could be related to fluoride intake.

A correlative chemical study on the same bone specimens has been made by Dr. I. Zipkin. In this study, as the fluoride concentration in the drinking water increased, the concentration in the bones also increased. Although very considerable

amounts of fluoride were deposited in these bones, the skeletal system was not adversely affected by a high fluoride content. These results furnish substantial additional evidence of the safety of water fluoridation as a public health measure for the partial control of dental caries.

CHEMICAL STUDIES OF DENTIN AND ENAMEL OF TEETH

It has been known for some time that the ingestion of fluoride results in a large measure of protection against dental caries. Just how this fluoride has been used by the body to produce this result has been the subject of much speculation. An increased content of fluoride in teeth does accompany the use of a fluoride water, as shown some time ago by Drs. R. C. Likins and F. J. McClure.

Mr. H. G. McCann has studied the dentin and enamel of teeth to determine the chemical reaction which occurs between the fluoride ion and tooth substance. He has established that the fluoride ion combines with hydroxyapatite, the chief mineral component of dentin and enamel, to form a fluorapatite.

To do this he first used a synthetic hydroxyapatite to study the chemical action of this compound and fluoride. Then using powdered human dentin and enamel to simulate the reaction which may occur when the teeth of an individual are exposed to fluoride, either by drinking water or topical application, he found that the reactions were more complicated than when the synthetic material was used. Relating these findings to those of a third study in which he found that the amount of fluoride deposited in bone, dentin, and enamel depended on the kind of tissue involved, he was able to postulate that there is a strong probability that the main product of the reaction between calcified tissue and fluorides in the body is the formation of fluorapatite. This may explain, in part, why teeth of individuals living in fluoride areas are more resistant to decay.

STANNOUS FLUORIDE STUDIES.

The use of stannous fluoride in place of sodium fluoride has been tested by various investigators in both laboratory and clinical studies with evidence of some additional benefits to be derived from the use of stannous fluoride. In a number of tests of the topical application of fluorides, the preliminary results with stannous fluoride suggest even greater effectiveness than with sodium fluoride.

Dr. J. C. Muhler and his associates at Indiana University have conducted a number of experiments. However, because of the instability of stannous fluoride, there are still technical problems to be solved before it could be used in place of sodium fluoride. Dr. Muhler is currently pursuing related problems under a grant from NIDR.

BONDED FLUORIDE STUDIES

Certain compounds of fluoride have been demonstrated to be without effect on either the incidence or severity of dental caries. The knowledge that the administration of one of these compounds to experimental rats resulted in no increase in the fluoride content of skeletal or dental tissues, led Drs. Likins and Zipkin to study the distribution of this compound in the body of the rat and to determine its pathway of excretion.

Using radioactive tracers, they found that potassium hexafluorophosphate could not be detected in the blood after 18 hours and at the end of 24 hours it was completely excreted in the urine. This phenomenon may explain the lack of effectiveness in reducing dental caries. The physical stability of this compound, however, coupled with its complete excretion, suggests its use as a research tool in kidney function studies.

AMERICAN MEDICAL ASSOCIATION'S RESOLUTION ON FLUORIDATION

Following a one-year study by two Councils of the American Medical Association, the House of Delegates adopted a report presented by its Reference Committee on Hygiene, Public Health and Industrial Health, endorsing fluoridation of public water supplies as an effective, safe, and efficient way of substantially reducing tooth decay.

NUTRITION AND DENTAL HEALTH

CHANGING CONCEPTS OF THE CAUSES OF TOOTH DECAY

There is general agreement, among most investigators, that refined sugars and certain minerals have essential roles in the process of tooth decay. It is becoming increasingly important however to also evaluate the role of protein, and, perhaps the effect of amino acid imbalances, as dietary factors involved in the etiology of tooth decay.

Recent studies by a grantee, Dr. L. A. Bavetta at the University of Southern California, and Dr. McClure and associates of the Dental Institute, demonstrated that a high incidence of

relatively severe dental decay is produced in white rats receiving diets containing 13% casein but little or no decay when the diet contained 24% casein. Caries-inhibitory effects of L-lysine, an essential amino acid, were also demonstrated using highly purified lysine-deficient diets.

In resolving the causes of dental caries there is need to investigate factors beyond those confined to the oral cavity. It is of particular interest therefore that a definite reduction in tooth decay in white rats was brought about by administering the amino acid, lysine, by stomach tube, thus bypassing the mouth. In addition it has been observed in recent experiments, that a certain chemical, dehydroacetate, also can be given to white rats by stomach tube, as well as by intraperitoneal injection, and cause a definite increase in caries.

Since these agents become effective without any apparent direct involvement within the oral cavity, the results furnish evidence that systemic metabolic factors are involved in increasing, as well as decreasing, the development of this experimental dental caries.

The value of certain mineral phosphates as caries preventive agents is currently under study in at least one institution, in addition to the National Institute of Dental Research. Studies with experimental animals at NIDR and also at Malmo, Sweden, have exhibited striking evidence that a definite caries inhibitory effect occurs with the presence of a phosphate mineral supplement in the diet. Phosphates are not unusual ingredients in flour, and therefore offer the possibility for human studies in caries control.

CARBOHYDRATE STUDIES

While it appears well established that carbohydrates, as an energy source for oral bacteria, are an important factor in initiation of dental caries, Drs. R. R. Steinman and M. I. Haley, grantees at the College of Medical Evangelists, reported differences between various sugars when these were incorporated into the diet of white rats. Significantly less caries occurred in the animals fed glucose or fructose as compared to those fed sucrose. This points to the possibility of dietary carbohydrate modification as a means of preserving oral health.

MULTIPLE FACTORS IN PERIODONTAL DISEASE

In attempting to meet the rapidly growing problem of periodontal disease, a natural outgrowth of today's aging population, Drs. S. S. Stahl,

S. C. Miller and E. D. Goldsmith, grantees at New York University, showed that periodontal disturbances can be experimentally created in animals both by induced malocclusion and by protein deprivation. This strongly suggests the importance of both proper chewing and proper diet in maintaining gingival health.

SYSTEMIC DISEASE AND ORAL HEALTH

EFFECTS OF LEUKEMIA AND ANTI-LEUKEMIA DRUGS ON ORAL TISSUES

A current study by Drs. J. H. Duffy and E. J. Driscoll, Clinical Investigations Branch, of 38 leukemia patients has resulted in the accumulation of valuable baseline data. Since leukemia patients on drug therapy must frequently be referred for dental treatment because of severely bleeding gums and other oral complications, they present special problems for both the dentist and physician. Of the 38 patients periodically examined, 80% had positive oral disturbances. Only those patients without teeth were free of these findings. The mouth lesions included non-specific gingivitis, hypertrophic gingivitis, frank bleeding, ulcerations and petechiae. In addition, it was noted that antimetabolite drugs produced characteristic oral lesions resembling canker ulcers. Further studies are contemplated to formulate prophylactic and treatment measures.

DEVELOPMENT OF ORAL STRUCTURE

STUDIES WITH THE ELECTRON MICROSCOPE, ELECTRON DIFFRACTION EQUIPMENT AND THE X-RAY MICROSCOPE

Progress in the study of dental tissues by these methods begins with the development and perfection of new techniques and tools. This work at NIDR is done jointly with the National Institute of Arthritis and Metabolic Diseases.

The use of carbon replicas of surfaces to study calcified tissues at high magnification has been found to be extremely valuable not only to provide information about tooth structure but has been demonstrated by Dr. D. B. Scott, Laboratory of Histology and Pathology, NIDR, and Dr. R. W. G. Wyckoff, Laboratory of Physical Biology, NIAMD, and their associates, to be useful in the study of a great variety of other material not only in the biological, but also in the physical sciences. The dental studies have resulted in the production of clearly defined photographs of submicroscopic crystals of tooth enamel, which, in the present stage of research on this portion of the

tooth, is most important in determining in detail the crystal size, shape, orientation and manner of development.

In studies with electron diffraction, because of the extremely small size of the prism, the basic structural unit of tooth enamel, it is necessary to be able to confine the area under investigation to as minute a section as one square micron. (The head of a pin would contain about 750,000 square microns). Earlier work with either electron or X-ray diffraction had not permitted this detailed exploration due to the large area covered by the illuminating beam. It is now possible as a result of work done in this laboratory to thoroughly scan the interior of a single enamel prism as well as the narrow region between prisms.

These laboratories have more recently reported on newly devised methods of producing X-ray microradiographs of soft tissue sections by the use of magnesium foil as target and creating a vacuum between target and photographic plate.

DEVELOPMENT OF MODIFIED MICROTOME

Dr. Marie U. Nylen, who is a Visiting Scientist from Copenhagen, Denmark, working in this laboratory, has perfected a modified microtome which will produce high quality ultra-thin sections for electron microscope study. This development is one result of a year of painstaking effort to secure an instrument which would cut sections of dental tissues one one-hundred thousandth of a millimeter in thickness. This accomplishment is permitting the study of fine structures of cells which form enamel and dentin, tracing the development of these tissues from the earliest stages to maturity.

ENZYME ACTIVITY OF DEVELOPING BONES AND TEETH

Dr. M. S. Burstone has demonstrated esterase activity in developing bone matrix. This has been accomplished by the use of a new substrate synthesized in this laboratory. This esterase has been found in both bone and bone tumors and appears to be related to calcification. Enzyme activity of bone matrix has not previously been demonstrated by histochemical procedures.

Dr. H. M. Fullmer from the same laboratory provided additional information on the growth and development of teeth in a study using human embryos in various stages of development. He demonstrated histochemical changes in tooth forming cells which occurred coincident with changes of function in these cells. These observations will aid in a better understanding of cell biology.

TOOTH GERM TRANSPLANTS

A grantee at Yale University School of Medicine, Dr. H. S. Fleming, has reported on the result of observations of growth and development of tooth buds (embryonic dental tissue) transplanted from guinea pig embryos. Recent studies have been directed toward observing the effect of various environmental conditions with the object of altering the growth pattern of the tooth bud. Previous studies by Dr. Fleming have shown that such transplants become a functional part of the hosts through connective tissue attachments and vascularization.

ORAL AND BIOLOGICAL CHEMISTRY

FORMATION OF PROTEINS

A study by Drs. K. A. Piez and Likins, Laboratory of Oral and Biological Chemistry, has contributed to the basic knowledge of the biosynthesis of collagen. Collagen is the major structural protein of many tissues including teeth, bone, tendon, and skin. The investigators, using radioactive carbon, have followed the conversion of lysine to hydroxylysine, a process that is believed to occur as part of an early stage in the synthesis of collagen. They showed that lysine is the only precursor of hydroxylysine and that the degree of conversion is different in different tissues of the same animal.

RADIOACTIVE STUDIES WITH AMINO ACIDS

Dr. Piez, in another study with Dr. H. Eagle, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, has discovered a unique isotope effect which is important in interpreting certain analytical results obtained with radioactive amino acids. They showed that amino acids labeled with radioactive carbon behaved differently when subjected to an ion exchange chromatography, a procedure used for the separation of amino acids. It was found possible to measure the isotope effect quantitatively and relate its magnitude to the position of the radioactive carbon atom in the amino acid molecule. The use of radioactive amino acids as tracers in studying biochemical processes, such as the biosynthesis of proteins, makes this an important finding.

HEREDITARY ASPECTS OF ORAL CONDITIONS

MICHIGAN AND MARYLAND STUDIES

Details concerning the part played by heredity in the

development of teeth and their supporting structures have been brought to light by Dr. C. J. Witkop, Jr., and his associates in the Clinical Investigations Branch, in large scale human studies which have been underway for the past two years.

Among the dental findings in two population groups is the greatly increased amount of dentinogenisis imperfecta or opalescent dentin found in an inbred group. A normal population study of children in Michigan, by Dr. Witkop, revealed the presence of this abnormality once in eight thousand persons. In contrast, in the inbred population group now being studied in Maryland, it occurs once in every thirty-five children. The teeth in such instances are usually amber brown in color with an opalescent hue. Some persons with this defect have "shell" teeth containing very large pulp chambers surrounded by a thin layer of dentin. To date eight distinct hereditary defects of enamel and dentin have been discovered.

Also under study in this group at the present time are a number of oral conditions related to speech abnormality. These include, tongue tie, cleft palate, malocclusion and malformed teeth, all of which are indicated as the cause of various speech defects. A study is currently being conducted to evaluate therapeutic benefits of surgical correction and speech therapy. The speech clinic at the University of Maryland is cooperating in this work.

The rich harvest of this entire genetic study is being cooperatively projected with scientists from the other Institutes of the National Institutes of Health. The prevalence of such abnormalities and diseases as albinism, anemia, deafness, and eye disturbances (glaucoma), as well as a variety of other defects, has been shown to be very high in this inbred group. These conditions are being studied for the purpose of determining the mechanisms of inheritance of the known hereditary diseases as well as to evaluate the role of heredity in other diseases not generally considered to be genetic.

DEVELOPMENT OF CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

Important grant supported experiments by Drs. H. R. Hunt, C. A. Hoppert and associates at Michigan State University, have included the development of two strains of rats, one caries-susceptible and the other caries-resistant. The susceptible animals show dental caries at approximately 70 days of age and the resistant ones at about 585 days.

From these two strains the Michigan group have conducted investigations in caries control which have yielded evidence of

greater frequency of certain bacteria such as lactobacilli and Streptococcus salivarius in the caries-susceptible rats. Enzyme studies of the saliva of these two strains of animals have been reported by this group of investigators which shed some light on the possible influence of protease on caries activity. These colonies of rats have also been the source of experimental animals used by scientists in other institutions for additional studies.

ORAL BACTERIOLOGY

IDENTIFICATION OF LACTOBACILLI

Lactobacilli have been generally recognized to play an important role in human and animal tooth decay. M. Rogosa and his associates in the Laboratory of Oral Bacteriology, have identified the lactobacilli in certain experimental animals and have also demonstrated the presence of seven hitherto undescribed species. This work now permits more controlled and more certain experiments on the relation between lactobacilli and dental caries in germ-free and other experimental animals.

THE RELATION OF BLOOD STREAM INFECTIONS TO ORAL FLORA

An important collaborative endeavor by Mr. Rogosa, Dr. T. A. Nevin, and Dr. E. G. Hampp, Senior Research Associate, American Dental Association, at NIDR, has been the study of blood stream infections observed after dental treatments. The importance of the study derives from the possible connection with endocarditis. The investigators have been able to detect the presence of organisms in the blood in 80% of the patients undergoing tooth extractions or extensive gingival curettage. This has been made possible by improving the culture media and the sampling techniques. In addition to their direct application to the problem at hand, these advances in blood culturing technique will doubtless be applicable to the study of other blood stream infections.

STUDIES WITH GERM-FREE ANIMALS

Other studies in this laboratory have been concerned with developing germ-free animal techniques for use in dental studies. It is hoped by this method to learn the part played by the separate bacterial strains present in the mouth and their interaction on oral health and certain aspects of general health. The excellent exploratory work done at Notre Dame (Lobund) indicates that this method of approach is

most promising. Among the Institute's investigators there is a long background of experience in dealing with the production of experimental caries in rats, both from a bacteriological as well as a nutritional standpoint. In addition to these activities a grant has been made to Dr. Frank Orland, University of Chicago, who is utilizing the Notre Dame facilities to study the role of bacteria in periodontal disease.

TREATMENT PROCEDURES

EVALUATION OF BIOLOGIC EFFECTS OF HIGH-SPEED AND ULTRASONIC DENTAL DRILLS

The biologic response of dental tissues to various types of high-speed and ultrasonic drills was evaluated by Drs. J. J. Kennedy and N. Buckman, Clinical Investigations Branch, in collaboration with workers from the U. S. Naval Dental School. Studying the effects of wide ranges of rotary instrument speeds (6,000 to 150,000 r.p.m.'s) on guinea pigs, it was found that only minor damage was caused in their erupting incisors. On the other hand, major damage was noted in the teeth and periodontal soft tissues of animals exposed to an ultrasonic instrument operating at 40,000 cycles per second. This damage consisted of arrested and aberrant dental growth, missing teeth, exfoliation of submandibular hair, and death. The least damaging effects were from water turbine machines; airbrasive units, and conventional rotary appliances operated at 6,000 r.p.m.

Other studies by Dr. H. Swerdlow and Dr. H. R. Stanley of this same Branch, reported the reaction of human dental pulps to cavity preparation using high-speed instruments at 20,000 r.p.m. Selecting noncarious, non-infected, vital teeth indicated for extraction because of periodontal and prosthetic problems, it was observed that extensive damage occurred when the cutting operation was completed without a water coolant. On the other hand, teeth prepared with a water spray showed histologic evidence of resolution or repair. A more complete biologic evaluation of the above instruments must await a better understanding of the innumerable factors involved.

CLEFT PALATE, MALOCCLUSION, AND OTHER CONGENITAL ABNORMALITIES

In relieving the serious physical and mental handicaps related to cleft palate defects, Dr. R. F. Hagerty, Medical College of South Carolina, reported promising results from the use of a special palatal bar for supporting the alveolar ridges in compression until the period of early ossi-

fication is completed. Results of the first year's use of the appliance show great possibility for compensating the cleft condition during the growth period prior to institutior of orthodontic therapy.

An important aspect of diagnosis and treatment of malocclusion impairment is the radiographic examination. Only by such early studies can the common impairments of mastication and speech be adequately analyzed. However, the possible radiation hazard from such analysis caused Drs. H. M. Berry, Jr. and F. A. Hofmann of the University of Pennsylvania, to devise an apparatus whereby motion pictures of the functioning temporomandibular joint could be made without any danger to the patient from excess radiation. Light multiplication techniques for image intensification, with the addition of photofluoroscopy methods, have enabled these investigators to report data of great value for analysis of both anatomical structure and physiological motion.

EPIDEMIOLOGICAL STUDIES

PERIODONTAL DISEASE AND CARIES

A system of classification and scoring for prevalence surveys of periodontal disease or pyorrhea, developed by Dr. A. L. Russell, Epidemiology and Biometry Branch, has furnished the framework upon which extensive epidemiological studies can be made. In one series of interesting studies, it was shown that fluoridated domestic water had no effect on periodontal disease. Other studies in 25 rural counties of Indiana gave evidence of an inverse correlation of periodontal disease with the general level of education of adults; i. e., the higher the educational level the lower the severity of disease. In contrast, urban populations, examined in a number of other states, showed less prevalence of disease than rural groups. Although these observations also suggested some racial differences, the evidence, at present, is quite inconclusive.

Another study by Drs. I. N. Hill and J. R. Blayney, grantees at the University of Chicago, showed a statistically significant difference in susceptibility to caries between white and Negro children. In this survey, white boys and girls in the 6-8 year and 12-14 year age bracket showed a greater susceptibility to dental decay than did Negro boys and girls of similar age, despite the fact that all 8,000 children examined resided in the Evanston, Illinois area which has had fluoridated water since February, 1947. It should be emphasized, however, that despite this difference in incidence, all children benefited most significantly from the fluoride.

HIGHLIGHTS OF PROGRESS

IN

MENTAL HEALTH RESEARCH

1957

Items of Interest on Program Developments and Research Studies Conducted and Supported by the National Institute of Mental Health

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The year 1957 has been one of noteworthy advance in mental health research. Significant progress has been made in a number of different areas: investigation of the biochemistry of the body as it affects the functioning of the nervous system, study of the operation and activities of the nervous system, research into the nature and bases of human and animal behavior, study of the effects of a variety of drugs on physiological and psychological function, and research into the effects on human beings of the social environments in which they live. In addition, there have been during 1957 a number of program developments which will play important roles in the implementation of research findings in the mental health field.

MOLECULAR DISORDER IN DISEASE

The last few years have witnessed a rebirth of interest in the many complex relationships between mind and body. Significant correlations between neuron and thought, between physiological change and behavioral change are being discovered. The ultimate general answers to the perplexing problems of these relationships may not be found for many years. Meanwhile, however, a multitude of specific questions are being answered almost daily by psychologists, physiologists, psychiatrists, biochemists, neurologists, pharmacologists, and workers in many other fields of scientific investigation. It is well understood, for instance, that psychological stress can be partly or wholly responsible for a number of physical ailments, among them hypertension, ulcerative colitis, certain types of asthma, and many others. Conversely, certain forms of mental illness and retardation have been shown to be rooted in physical disease or dysfunction. Paretic psychosis, arterio-

sclerotic psychosis, galactosemia, and phenylketonuria are some of the mental abnormalities in this class.

In view of the rich possibilities and exciting leads in this field of investigation, scientists all over the country are now undertaking a variety of approaches to the study of body-mind relationships. Basic to these studies is an accurate and intimate understanding of the many subtle and complicated metabolic processes of the body, an understanding which is being achieved by the use of highly sensitive new as well as classical qualitative and quantitative biochemical techniques.

BIOLOGICAL BASES OF SCHIZOPHRENIA

Prominent among the studies of the biological aspects of mental illness is a project being launched by the Laboratory of Clinical Science, NIMH, under the leadership of Dr. Seymour S. Kety. This study will test many of the hypotheses which postulate some characteristic biological changes related to the schizophrenic disease process.

Although investigators have been reporting a great variety of anatomical and physiological abnormalities in schizophrenics for over a half century, most reported results have not been substantiated by later, better controlled studies. A number of studies, however, do present evidence that schizophrenia, or a predisposition to it, is, in part at least, genetically determined. In addition, reliable physiological studies have shown that about 28% of the schizophrenic population show electroencephalographic abnormalities compared with only about 7% of normals. In addition, it is widely agreed that there exists a disturbance in the carbohydrate metabolism of schizophrenics, although there is a serious question as to how this disturbance is related to the illness.

Convincing evidence, produced chiefly by the Sections on Cerebral Metabolism and Psychiatry of the Laboratory of Clinical Science, indicates that, contrary to some previously held views, there is no abnormality either in cerebral circulation or total oxygen consumption of the brain in schizophrenia.

As a result of these and many other physiological studies of metabolism in schizophrenics, there are at present three general hypotheses concerning a basic biochemical cause of the disease. These hypotheses concern, respectively, abnormality in epinephrine, ceruloplasmin and serotonin metabolism.

Two factors explain the spotty and inconsistent results of research into the biochemical aspects of schizophrenia. First, the diagnosis of schizophrenia is apt to include a number of different diseases with common symptoms. This fact would introduce major errors in studies of relatively small patient

samples. The other problem is that there is a general failure to control the non-disease variables, which are either secondary symptomatic features of the disease or which are associated with the chronic hospitalization to which most of the patients are subjected. Metabolic abnormalities which result from inadequate or poorly balanced diets of hospitalized mental patients illustrate this class of experimental flaws.

Poorly supported though they are, however, the theories of a biochemical basis for schizophrenia are provocative enough to have stimulated the Laboratory of Clinical Science to set up a long-range, broad scale, controlled program of investigation. About 15 carefully screened and selected schizophrenic patients and the same number of picked normal controls will be used in the program. Both schizophrenic and normal populations will be maintained as much as possible under similar conditions of diet, activity and management. Using classical biochemical techniques, supplemented by new and sensitive techniques for the detection, tracing and quantification of chemical substances in minute amounts -- chromatography, isotopic tracers, ultraviolet, infrared, and fluorescent spectrophotometry -- the members of the Laboratory expect to test the most important and promising hypotheses relating to the possible biochemistry of schizophrenics. Among the phenomena which will be studied and correlated in the two subject populations are EEG changes; the effect and metabolic fate of epinephrine; blood levels and metabolism of glutathione, copper, ceruloplasmin, and ascorbic acid; and the metabolism of such amino acids as tryptophane, histidine, glutamine and tyrosine. It is hoped that this carefully set up and rigidly controlled program of investigation will help to eliminate the erroneous hypotheses about biochemistry and schizophrenia, and will bring us closer to a knowledge of what, if any, biochemical phenomena actually do underlie the disease process.

TEST OF CERULOPLASMIN HYPOTHESIS

During the past year, Dr. Roger McDonald of the Laboratory of Clinical Science cast some serious doubts on the theory that faulty ceruloplasmin metabolism underlies schizophrenia. Early this year it was reported that fresh serum of schizophrenic patients had the capacity to oxidize a dye, N,N-dimethyl-p-phenylenediamine, more rapidly than did the fresh serum of healthy, normal subjects. This more rapid oxidation was taken to mean that schizophrenic patients had higher than normal blood levels of ceruloplasmin, and that this, conceivably, was related to the psychotic process. Dr. McDonald has demonstrated, however, that the rapidity of the oxidation reaction is more closely related to serum levels of ascorbic acid than of ceruloplasmin, and that differences between psychotic patients and normal controls probably reflect a dietary rather than a pathological

phenomenon. Schizophrenic patients at the Clinical Center of the National Institutes of Health, for instance, being on a carefully balanced and supervised dietary regimen, show serum levels of ascorbic acid about the same as those of normal subjects. The fresh serum of these patients consequently fails to oxidize the dye more rapidly than does the serum of representative normal controls.

TEST OF SEROTONIN HYPOTHESIS

Dr. Harris Isbell of the NIMH Addiction Research Center, Lexington, Kentucky, has carried on a series of studies which question the validity of the theory that a deficiency of serotonin in the central nervous system is the cause of psychosis in man. Dr. Isbell has tested a number of drugs which are similar to one another in that they reduce the level of serotonin in the nervous system, and has discovered that the behavioral effects of these drugs on human subjects are quite different from one another. Since there appears to be no correlation, therefore, between the ability of a drug to lower nervous system serotonin and to produce psychotic symptoms, some doubt has been cast on the serotonin theory of psychosis. The fact that this experiment did not support the theory that serotonin deficiency causes psychosis does not mean, however, that the theory has been disproved, since any one of a number of yet unknown factors may be responsible for the negative results. It does mean that the theory needs further careful and cautious study and testing.

METABOLISM OF EPINEPHRINE

New light has been cast by a NIMH scientist upon the metabolism of epinephrine and norepinephrine, the derangement of which has been postulated as a psychosis-causing phenomenon. Dr. Julius Axelrod, Laboratory of Clinical Science, has discovered a new enzyme responsible for the metabolism of 70% of these two substances in the body in the rat. In addition, his discovery discloses a hitherto unknown metabolic pathway for the transformation of a variety of body metabolites.

Epinephrine and norepinephrine are both intimately concerned with the functioning of the nervous system, playing important roles in the response of the organism to stress -- the "fight or flight" reaction, in the regulation of blood pressure, of carbohydrate metabolism, and other nervous system activities. It is conceivable, then, that a disorder in the metabolism of epinephrine and norepinephrine, intimately concerned as they are with nervous system function, could be responsible for the development of psychotic symptoms. Since the understanding of the behavioral effects of such metabolic disorders must depend upon a prior knowledge of normal epinephrine and norepinephrine metabolism, Dr. Axelrod's work is of great

significance in laying the groundwork for future research.

THYROID FUNCTION AND BEHAVIOR CYCLES

In an attempt to understand the periodic cycles in behavior, mood, and metabolism often seen in the mentally ill, NIMH grantee Dr. Curt Richter, The Johns Hopkins University, has carried on a series of experiments with rats as subjects. Theorizing that thyroid gland function is somehow involved in these alternations of relatively normal and abnormal activity, Dr. Richter and his associates interfered with the normal functioning of the rats' thyroids by surgical, chemical, and pharmacologic means. In about one-third of all rats whose thyroids were either partially removed, partially destroyed by chemicals, or rendered less active by drugs, regular behavioral cycles appeared. These cycles corresponded in many ways to the alternations of grossly psychotic and relatively normal behavior which many psychiatrists have noted in schizophrenic patients. The cycles were apparent in changes in the animals' spontaneous activity, and, in some of the animals, by changes in food and water intake and in body weight. The ability to produce regular behavioral cycles experimentally in animals should lead to a deepened understanding of the phenomena underlying the behavioral cycles so often observed in psychiatric patients.

AMINO ACID METABOLISM

In a study of the metabolic defect which underlies phenylketonuria, Dr. Seymour Kaufman of the Laboratory of Cellular Pharmacology, has discovered that a hitherto unknown co-enzyme is necessary for the proper metabolism of phenylalanine, an essential amino acid. One of the symptoms of phenylketonuria is severe mental retardation.

In earlier studies of the complex conversion of phenylalanine to tyrosine, Dr. Kaufman had discovered that the reaction required the presence of at least two enzymes, oxygen, and a co-enzyme, TPNH (reduced triphosphorpyridine nucleotide). In the course of further purification of one of the enzymes from rat liver homogenate, Dr. Kaufman has discovered that another co-enzyme besides TPNH is required for the conversion.

The new co-enzyme has not yet been identified, but it appears to be different from any known vitamins and co-enzymes, none of which can replace it in the phenylalanine to tyrosine reaction. The co-enzyme is not widely distributed, since it fails to appear in trial extracts of such tissues as beef spleen, heart, brain, thyroid, kidney, pancreas, rabbit muscle, in brewer's yeast, baker's yeast, or in several other extracts. Only beef adrenal glands and all liver extracts so far tested appear to contain it.

The fact that the new co-enzyme activates the conversion from phenylalanine to tyrosine only in the presence of TPNH suggests that it operates by means of enzyme-catalyzed reaction between TPNH and itself.

What makes the phenylalanine to tyrosine conversion especially interesting biochemically is the fact that it is a rare true oxidation, in which oxygen is added to one of the reacting molecules to produce the end product, rather than the more common oxidation, which is, more exactly, a de-hydrogenation of one of the reacting compounds.

Dr. Kaufman's discovery increases our knowledge of amino acid metabolism in general, and also gives us a deeper understanding of the metabolic error responsible for phenylketonuria. With this understanding, researchers can intensify efforts to improve dietary therapy for children suffering from phenylketonuria.

BASIS OF ALLERGIC ENCEPHALOMYELITIS

In another attempt to probe the biochemical processes underlying the diseases of the brain and nervous system, Dr. Marian W. Kies of the Laboratory of Clinical Science, NIMH, in collaboration with Dr. E. C. Alvord, Jr. and Dr. Elizabeth Roboz, has been searching for the substances responsible for the production of allergic encephalomyelitis, an inflammation of the brain and spinal cord.

For many years, allergic encephalomyelitis has been produced experimentally in animals by injection of a mixture of whole brain or spinal cord and killed tubercle bacilli. Through the work of Dr. Kies and her colleagues, it now seems probable that the specific compounds within the spinal cord and the killed bacilli preparations which are responsible for the disease will be found.

Using their own preparations of spinal cord and the bacilli preparations of Dr. J. Colover, Taplow, England, Dr. Kies' group has shown that the whole killed tubercle bacillus and whole brain or cord is not necessary for the production of the disease. They have shown that fat-free and protein-free tissues are capable of producing allergic encephalomyelitis, and have taken significant steps toward the production of pure compounds in the fractionation of cord and bacilli.

This work makes it appear inevitable that the crucial experiment -- production of the disease with two pure chemical compounds -- will eventually be performed. When this is done, it will provide final information on the specific nature of the sensitizing process responsible for allergic encephalomyelitis.

STUDY OF THE NUCLEIC ACIDS

The Institute's most basic work on the chemistry of the body is being pursued in the Section on Physical Chemistry, under the direction of Dr. Alexander Rich. This section is carrying on a continuing study of the molecular architecture of ribonucleic acid (RNA), believed to be the fundamental substance responsible for protein synthesis in the cell and, along with deoxyribonucleic acid (DNA), for the transmission of genetic information.

The members of the section have concentrated their study on synthetic polyribonucleotides which, while yielding accurate information about molecular structure, are simpler to deal with than the naturally occurring molecules. Last year it was discovered that one synthetic molecule exists in the form of a two-stranded helix, with polyadenylic acid and polyuridylic acid strands connected to each other by hydrogen bonds. During the past year, Dr. Rich and his colleagues have discovered that it is possible to make a three-stranded nucleic acid molecule. This development may have important implications in revealing to us the way nucleic acid molecules transmit information. Conceivably, the transfer of information is accomplished by the dictation of a specific nucleotide sequence from a two-stranded DNA molecule to a third molecular chain as it synthesizes a three-stranded RNA molecule.

These and other studies of the section have now permitted the investigators to deduce a total of eight different structures which will form from the synthetic polynucleotides. These structures provide a basic set of information which will now help in revealing the configuration of naturally occurring RNA in cellular systems.

The discoveries of this laboratory will undoubtedly be of great significance in helping us to understand the fundamental chemical processes underlying cell growth, inheritance, and metabolism.

CELLS AND SYSTEMS

A number of advances have been made during the past year in the study of the activities of nerve tissue. We are achieving a clearer conception both of the elemental structure and the normal and abnormal functioning of the nervous system.

STUDY OF THE ELECTRICAL ACTIVITY IN SINGLE NERVE CELLS

An investigator in the Laboratory of Neurophysiology has developed a new method of studying electrical potentials in the body and dendrites of nerve cells, which has

already revealed that this portion of the nerve cell does not itself produce self-propagating electrical activity.

Early studies of the physiology of the nervous system were confined chiefly to the axons - long fibers which serve to conduct impulses from one nuclear mass of the nervous system to another. These axons were found to operate on an "all-or-none" basis; either a standard and unvarying response was made, or the axon failed to conduct any impulse whatever. As a result of these studies, investigators assumed that the "all-or-nothing" response was true of the entire neuron.

More recently, a number of neurophysiologists have discovered that the electrical activity of the nerve cell body and its branching dendrites is much more complex than was earlier supposed. Studies performed by placing recording pipettes within the cell body have shown that a variety of graded responses occur at the synapses of the nervous system. A great number of stimuli may be received by the cell body, which, after responding to them in a cumulative fashion, codes them into an "all-or-nothing" response for long distance transmission along the axons.

One problem which has plagued investigators recording electrical activities from within the cell body is that they can never be sure that electrical potential is produced across the part of the cell membrane the pipette happens to enter. In addition, it has not generally been possible to place pipettes within dendrites to record the electrical activity occurring there.

A new technique, developed by Dr. Walter Freygang and his associates in the Laboratory of Neurophysiology, NIMH, now permits investigators to measure membrane current by means of pipettes placed just outside the membrane of the nerve cell body and dendrites. Although the soma-dendritic membrane does respond to chemical excitation, measurements of membrane current have shown that it is incapable of producing "all-or-none" propagated activity. This technique avoids some of the unsureness of older techniques of recording, and gives promise of permitting physiologists to understand and assess more fully the fundamental changes in nerve-cell membrane permeability which occur during synaptic activity, and which explain more adequately the mechanism of action of the graded response.

AGE CHANGES IN NERVE TISSUE

A problem which is receiving much attention today from neurophysiologists and neuroanatomists concerns the changes which nerve tissue undergoes during the process of aging. Many studies have indicated a variety of age changes in nerve cells, but most of these

have turned out to be not age changes at all, but either pathological changes or artifacts, i.e., alterations caused by the very fixing and staining procedures necessary for accurate microscopy. While there has been general understanding for many years that these artifacts seriously affect our microscopic picture of tissue, the tremendous technical problems involved in trying to study tissue in any other way have hindered substantial advances. There is still no way known to study nerve cells under the microscope without introducing changes in them. Dr. William Bondareff, of the Section on Aging of the Laboratory of Psychology, however, has been working to improve ultra rapid freezing and drying techniques in the preparation of nerve tissue for study with the light and electron microscopes, in an attempt to discover whether, in fact, non-pathological changes actually do occur in tissue as it ages. Dr. Bondareff's frozen-dried tissue does not contain ice crystals, as some earlier frozen preparations did, and hence permits him to interpret the micrographs with increased accuracy. Preliminary results of his work indicate that lipofuscin, the pigment which has been widely observed in certain aging nerve cells, while probably not directly related to the aging process, may still be used as an index of cellular aging.

EPILEPSY AND THE CONTINUOUS PERFORMANCE TEST

Increased understanding of the normal and abnormal functioning of the nervous system may result from a finding that patients suffering from focal epilepsy function significantly better on a test requiring sustained attention and vigilance than do patients with some types of non-focal epilepsy. Apparently, epilepsy-producing disturbances or disease in certain areas of the cerebral cortex do not interfere with the ability to concentrate, while a generalized (possibly sub-cortical) disturbance does affect the ability to maintain continuous attention and performance. This finding will be of value in the formation of surer theories about the precise mechanism underlying some types of non-focal epilepsy. Responsible for this investigation are Dr. Allan F. Mirsky and Daniel Primac of the Section on Animal Behavior, Dr. Cosimo Ajmone Marsan, NINDB, and Janice R. Stevens, of the University of Oregon Medical School.

NEW SECTION ON LIMBIC INTEGRATION AND BEHAVIOR

An important addition was made this year to the Laboratory of Neurophysiology in the form of the Section on Limbic Integration and Behavior, headed by Dr. Paul D. MacLean, internationally recognized neurophysiologist. Dr. MacLean's work has been principally in the areas of psychosomatic and emotional disorders and their neurophysiological correlates. Dr. MacLean's research in this field has led to the development of important theories relating

the mechanism of the limbic cortex to unconscious visceral activities involved in some psychosomatic illnesses and emotional imbalances.

NATURE OF RESPONSE TO BRAIN STIMULATION

Dr. John Lilly, of the Section on Cortical Integration of the Institute's Laboratory of Neurophysiology, has demonstrated during the past year that electrical stimulation of certain parts of the brain appears to produce a true emotional state in unanesthetized monkeys.

For years apparent emotional reactions of various sorts have been observed in monkeys when they were stimulated through electrodes implanted in their brains. Most investigators, however, thought that the terror response to stimulation of the hypothalamus, for example, was simply an activity of the peripheral motor mechanism, and did not represent truly "felt" emotions. Dr. Lilly, to test this theory, placed potentially damaging objects near the monkey and applied a very light electrical stimulation, far below the level that would produce convulsive activity. He found that during stimulation of a particular locus that apparently controls feelings of fear and terror, the monkey bit fiercely and repeatedly, so that teeth were broken out of the jaw. The biting continued until the object was removed or stimulation was stopped. The obvious pain of biting and breaking teeth is apparently less unpleasant to the monkey than the emotions of fear and panic induced by the stimulation.

In another experiment, monkeys forgot a response which switched off pain-causing stimulation sooner than they did a response which switched off the terror-causing hypothalamic stimulation. Apparently the emotion felt as a result of this stimulation is more unpleasant to the monkey than severe pain.

BRAIN STIMULATION AND DRUGS

In similar work, Dr. James Olds, working partly under an NIMH research grant, is using direct stimulation techniques for the analysis of drug effects on behavior. Experimental animals, it has been found, will repeatedly press a bar turning on an electric current which causes a pleasurable stimulation. After discovering areas in which the sensation is so pleasant that the animals will press the operating lever repeatedly and at high rates of speed, Dr. Olds administered a variety of drugs to discover their effects on the animals' desire for self-stimulation. So far he has discovered that chlorpromazine and pentobarbital inhibit self-stimulation of certain parts of the brain, but not of others. Lysergic acid, a hallucinogenic drug, briefly inhibits all self-stimulation.

Dr. Olds has perfected his technique to the point where the animals can cause minute amounts of various drugs to be injected into their own brains by pressing various levers. In this way, he can discover which drugs injected at which sites in the brain produce rewarding effects; that is, effects which cause the animals to administer it to themselves again and again. Already Dr. Olds has discovered that Marsalid (one of the "energizer" drugs) has a rewarding effect when injected into certain specific areas in the hypothalamus.

These techniques for the testing of drug effects hold great promise for enabling investigators to determine much more exactly where and how drugs affect the brain and nervous system.

HUMAN AND ANIMAL BEHAVIOR

Obviously, most forces which act upon the nervous system will show their effects in the behavior of the individual. Physical damage to the brain, drugs, and chemicals which act on the nervous system, may sharply modify behavior. Much more subtle factors also affect behavior and physiological processes as well.

HANDLING AND RESISTANCE TO STRESS

One theory holds that if animals are handled in infancy, their nervous systems will develop the ability to meet stress more successfully in later life. To test this theory, NIMH grantee Dr. Seymour Levine, Columbus Institute of Psychiatry, Columbus, Ohio, used two groups of rats, one consisting of rats that had been handled once daily for the first 20 days of their lives, and the other consisting of rats that had been left untouched. When the rats were 70 days old, both groups were subjected to the stress of being injected with a 20% solution of glucose and placed in individual cages without food or water for 24 hours. At the end of 24 hours, the rats were permitted to drink water freely for one hour.

Differing reactions of the two groups of rats suggest differences in the amounts of adrenocorticotropic hormone and antidiuretic hormone secreted. Both these hormones are involved in the response of an animal to various stresses. These differences in reaction to physiologic stress between the handled and non-handled rats support the theory that handling in infancy reduces the physiological reaction to stress. Since the stress reaction appears to be mediated through the central nervous system, Dr. Levine theorizes that being handled in early infancy somehow modifies the later reactivity of the central nervous system under stress conditions.

BRAIN AND BEHAVIOR

The relation between subcortical mechanisms of the brain and behav-

ior is being investigated by the Section on Animal Behavior of the Laboratory of Psychology. In one set of studies, Dr. H. Enger Rosvold and his associates have discovered some new relationships between damage in various areas of the brain and resultant behavioral deficits.

A number of these findings give evidence that many classical conceptions about which brain areas control which functions are either incorrect or inadequate. Other studies in this section show that social behavior of animals after they have sustained brain damage depends not only upon the location and the extent of the damage, but also upon the nature of the social environment before and after the operation.

BRAIN, SOCIAL ORGANIZATION, AND BEHAVIOR

Dr. Allan F. Mirsky and his colleagues set up a number of groups of monkeys for baseline study of their social organization and behavior. They observed the members of these groups competing for food, and for each group developed a picture of typical social interaction, which included aggressiveness and submissiveness. In each group a hierarchy developed, with some animals clearly and consistently dominant and others habitually dominated.

In general, after frontal lobotomy the lower-ranked animals in the social scale became less fearful. Dominant animals, on the other hand, became less aggressive after sustaining lesions of the temporal lobe.

Behavior of the animals whose temporal lobes were damaged, however, showed variations which could not be accounted for by the slight anatomical differences in the brain lesions. They seemed rather to be related to the nature of the hierarchy of which the particular animals were members. A monkey who was indisputably dominant, and in a group in which the other members tended not to compete with him, tended to remain dominant. On the other hand, an animal who was part of a hierarchy where close competition existed for the top spot tended to decline in dominance. In other words, the social experiences that the operated monkeys met on returning to their groups seemed either to intensify or limit the effect of the operations.

These studies may have important applications in the field of rehabilitation of individuals who have undergone brain surgery. The results could mean that the attitudes toward, and treatment of the patient after his return to his social environment might influence the way in which his personality is affected by the operation. Therefore, it is suggested that accurate predictions of behavior after brain damage cannot be made solely on the basis of the area of the brain which has been affected.

STUDY OF BEHAVIOR FREQUENCY AND DURATION

The "spontaneous" operations of the nervous system are under study by Dr. John B. Calhoun, Section on Perception and Learning of the Laboratory of Psychology. Trying to answer the question of why and how specific behaviors are triggered in the nervous system, Dr. Calhoun has devised an apparatus in which three separate behaviors of the rat -- sleep, eating, and exploration -- can be observed. Each animal under observation lives alone in a long, tunnel-like structure, at one end of which he performs his sleeping and eating behavior. He is completely free to explore any part of the structure or to eat or sleep any time he chooses for as long as he chooses.

Electrical counters placed at various locations in the structure indicate the type and duration of uninterrupted acts of sleeping, eating, or exploration.

Results show a constant and consistent relationship between the duration and frequency of single behaviors. Although they are uninterrupted by outside stimuli, the rats show a great preference for behavior of short duration. The fact that eating, sleeping, and exploring behaviors appear to be interrupted frequently by some apparently spontaneous action of the animal's nervous system leads the investigators to theorize about the existence of some internal pace-making or setting function of the central nervous system. Other studies by Dr. Calhoun indicate that animals with certain differences in genetic or environmental backgrounds have demonstrably different patterns of spontaneous nervous activity.

Graphically illustrated, the results of the experiments show that the frequency with which single behaviors occur decreases steadily as their duration increases. It is possible that similar graphs can be used to illustrate a variety of more complex behaviors of human beings. If they can, and if characteristic duration-frequency equations can be determined, this approach to the study of behavior may prove to have value in the description and analysis of normal and aberrant human behavior.

Human behavior, of course, is by far the most subtle and complex kind to analyze and comprehend. Still, investigators have devised a great variety of techniques designed to give them an increasing understanding of the sources of and motives for human behavior -- an understanding aimed ultimately at helping those people whose disorganized and inadequate behavior is symptomatic of mental illness.

PARENTAL ATTITUDES AND CHILD PERSONALITY

Dr. Earl Schaefer and Richard Q. Bell, Section on Child Development, Laboratory of Psychology, have spent a number of years in developing PARI, the Parental Attitude Research Instrument. This is a test for measuring a number of parental attitudes which the investigators believe are related to the ultimate social and emotional adjustment of children. They theorize that particular attitudes on the part of parents tend to produce children with characteristics discernibly related to these attitudes.

During the past year, evidence has been gathered which tends to support the belief that specific patterns of mother-child interaction can be predicted even before the birth of the child, by use of the PARI. Success in this sort of prediction would be of great value in the study of the influence of the parent-child relationship upon the child's personality development.

Drs. Schaefer and Bell administered their Parental Attitude Research Instrument to a control group of 100 student nurses in an attempt to discover whether clusters of attitudes shown on this test bear any similarity to syndromes of parental attitudes and behavior which have been clinically observed.

A relationship was revealed between attitudes shown on the test and clinically observed parental attitudes and behavior. This study, therefore, along with other similar ones, suggests that prediction of mother-child interaction and of personality development of children may be possible, since measureable variability in attitude and an organized structure of attitudes toward child rearing can be determined in young unmarried women.

National Institute of Mental Health scientists and grantees are studying human behavior at both ends of the scale of age. As some investigators attempt to discover the genetic and the earliest environmental forces instrumental in shaping and influencing the development of the child's personality, others are at work trying to find out what happens to people as they grow older -- how the cells of their bodies age, how the physiological changes of age affect their attitudes, how psychological age changes affect physical health.

GRANT FOR AGING RESEARCH

During the past year, the National Advisory Mental Health Council for the first time made a grant for the establishment of a research program designed to focus on all aspects of a broad

inter-disciplinary research problem. The grant was made to Duke University in collaboration with the National Heart Institute to set up the Center for Aging Research, under the direction of Dr. Ewald Busse. The Center will deal with social points of view, and will attempt to focus on all the problems of aging, with a realization of how intimately all are interwoven with one another. The specific aims of the Duke program are the following:

1. To develop a Center for Aging Research firmly built upon a University-wide integrated effort and utilizing, when appropriate, an inter-disciplinary approach.

2. To encourage and support fundamental research concerned with the phenomena and health problems of aging and to include relevant research contributions from the social and behavioral sciences and related fields.

3. To train investigators, and give them an opportunity to become familiar with and interested in problems of aging, and to acquaint them with the usefulness and limitations of an interdisciplinary approach.

4. To foster a regional resource which will have adequate facilities to disseminate useful knowledge concerned with the promotion of health and social adaptation of our aging population, and to provide for State and local government as well as for private groups and individuals a source of scientific knowledge in the field of aging.

NIMH intramural scientists have, during the past year, produced evidence which contradicts ideas of long standing about the relationship of aging and cerebral oxygen consumption. It has been widely held that the aging process is accompanied by a gradual but steady decrease in cerebral oxygen consumption. To test this belief, Dr. Louis Sokoloff and his associates in the Section on Cerebral Metabolism of the Laboratory of Clinical Science carefully screened and selected for study a group of aged men in whom age was uncomplicated by overt physical or psychiatric disorder. Selection of subjects was done in collaboration with Dr. James E. Birren, Chief, Section on Aging of the Laboratory of Psychiatry, and Dr. Seymour Perlin, Chief, Section on Psychiatry of the Laboratory of Clinical Science.

The cerebral oxygen consumption per unit time per unit weight of brain of this group, with a mean age of 71 years, was not significantly different from oxygen consumption in a group of healthy normal controls, with a mean age of 21. The results of this work mean that many traditional ideas of "normal" changes in cerebral metabolism with age will have to be revised. The fact that probably a great majority of older people do suffer from the common diseases of age which tend to reduce cerebral oxygen consumption does not affect the significance of the conclusion that aging, per se, does not cause a depression in cerebral metabolism.

Psychiatrists and neurophysiologists will now follow up these subjects, as well as a sample of aged men in whom cerebral oxygen uptake was lower, but still within normal limits. By continuing psychiatric evaluations and physiological measures of cerebral metabolism, the investigators hope to discover more about those factors responsible for a downward trend in cerebral metabolic rate with age, and to increase their ability to predict future psychiatric and physiological changes.

DRUGS AND THEIR EFFECTS

The past year has seen further developments relating to drugs which affect the mind and the emotions. Perhaps the two most important classes of these drugs are the tranquilizers and the energizers. The National Institute of Mental Health, in both its intramural and extramural programs, has continued to study and to evaluate these and other important drugs. Grantees and intramural scientists are at work synthesizing and isolating potentially active psychopharmacologic agents, investigating the biochemical bases of psychosis and the biochemical and metabolic mechanisms of drug action, making studies of possible toxicity or addictiveness in new drugs, and observing the behavioral and neuropharmacologic effects of the drugs in human subjects.

CHLORPROMAZINE AND THE EFFECTS OF FEAR

Important among these studies being carried forward is one which indicates that chlorpromazine achieves its tranquilizing effect by causing a change in psychological perception, rather than by inducing any sensory or motor deficit.

National Institute of Mental Health grantees Drs. Robert E. Miller, John V. Murphy, and I. Arthur Mirsky, University of Pittsburgh, have studied the effects of chlorpromazine on rats' avoidance responses to a stimulus which had previously been associated with shock. Their aim was to localize and quantify the nature of these effects.

They found, first, that the reduction in fear-motivated behavior is a function of the size of the dose: the larger the dose, the greater the reduction in fear-motivated behavior.

The second problem was to determine whether the extinction of the response--in this case a fear reaction to a buzzer--was due simply to a motor and/or sensory deficit caused by the drug, or to the effect of the drug on the psychological motivation of fear. Analysis of the rats' behavior by means of bio-assay statistics leads the researchers to conclude that the decrements in avoidance behavior are not attributable to sedative effects of the drug on sensory or motor nerve pathways, but are related, rather, to a change in the "psychological meaning" of the conditioned stimulus which the drug effects in the animal's brain.

STATISTICAL STUDY OF TRANQUILIZING DRUG TRIALS

A member of the Biometrics Branch of the Institute, in a statistical review of 25 separate studies evaluating the use of tranquilizers in mental

illness, has revealed an average decrease of over 50% in disturbed ward behavior and secondary symptoms. That other than purely pharmacologic forces are at work, however, is shown by an average decrease of about 20% in these symptoms in control patients given placebos.

In a group of trials of chlorpromazine analyzed by Mr. Samuel Greenhouse, average improvement rate for patients receiving the drug was 56%. Rate for controls receiving a placebo was 25%. The figures in a group of reserpine studies were 54% improvement rate for patients given the drug as compared to 18% for controls who received a placebo.

It is important to understand that improvement in this sense does not mean remission of the disease, but rather refers to favorable changes in behavior and symptoms secondary to the disease.

Noteworthy in these studies were (1) the fact that in most studies the drug group showed a significantly greater improvement than the control group, and (2) the relatively high improvement rate in patients given the placebos in many of the studies.

Mr. Greenhouse observes from his survey that difficulties appear to arise in maintaining the double blind procedure and in isolating various factors of the experiment which modify the hospital environment and behavior of the members of the staff.

TECHNIQUE FOR TESTING DRUG EFFECTS

The development of a new technique for testing drug effects in animals holds promise of great usefulness in the study of various sorts of behavior. This development is a modification of the classical choice-box for the testing of visual discrimination.

Dr. Donald Blough, Laboratory of Psychology, NIMH, has developed a variation of the standard Skinner Box, an apparatus designed to record a simple, repeatable response in a restricted and controlled environment. Dr. Blough's modification makes it possible to record two measures of response: the total response output, and accuracy of response. Intermittent food reinforcement maintains the response behavior of the pigeon who responds to a lighted key stimulus.

Visual discrimination of pigeons was tested in control situations and under the influence of five drugs: chlorpromazine, LSD-25, meperidine, caffeine, and pentobarbital. Results under influence of drugs as compared to those in the control situation were as follows:

DRUGS	MEASURES OF RESPONSE	
	Response Output	Accuracy of Response
Chlorpromazine (high dosage level)	Decreased	Decreased
LSD-25	Lowered at first	Increased
Meperidine (large dose)	Decreased	Decreased
Caffeine	Increased slightly	No effect
Pentobarbital	Increased sharply for short time	Decreased sharply

Possessing, as it does, the ability to record two different measures of response over a prolonged period, Dr. Blough's apparatus, when used in tests of drug effects, shows promise of being a most valuable tool for the analysis of the variables which control behavior.

POTENTIAL TOXICITY OF TRANQUILIZERS

In a study of possible toxic effects of chlorpromazine, investigators have discovered a potential hazard in the drug's tendency to cause bizarre and hallucinatory-type behavior and convulsions when administered at high dosage levels.

Drs. Carl F. Essig and Woodrow W. Carter of NIMH's Addiction Research Center, Lexington, have discovered that high dosages of chlorpromazine cause convulsive actions in monkeys with no history of epileptic seizure. The investigators observed that the use of chlorpromazine at high dosage levels may be hazardous in any number of situations, but that special hazards exist when any drug with convulsive properties is used in the treatment of epilepsy and the management of alcohol and barbiturate withdrawal.

The literature notes this effect in subjects with pre-existing epilepsy, but this is the first time it has been observed in normal animals. Four monkeys were started on a gradually increasing dosage schedule of chlorpromazine. In all the animals, the grimacing and aggressive behavior common to the Rhesus monkey was reduced, but tremulousness was noted in all the animals' extremities. No convulsions occurred at a dose of 25 mg./kg. of body weight, but each of the four monkeys suffered convulsions

at doses ranging from 44 to 77 mg./kg. a day. The total number of convulsions in each animal ranged from two to twelve. None of the animals was rendered permanently epileptic by this experience. Although there may be a species difference with respect to chlorpromazine, roughly comparable high doses of chlorpromazine have been given to human patients.

POSSIBLE MEPROBAMATE ADDICTION

Further evidence has been accumulated by workers at the Addiction Research Center that continued large doses of meprobamate can create physical dependence, shown on withdrawal from the drug by great irritability of the central nervous system and convulsions. The investigators note that addiction to meprobamate will probably occur in only a very small proportion of the drug users, but caution physicians to exercise great care in prescribing the drug, to write prescriptions which the patient cannot have refilled himself, and to withdraw the drug slowly rather than abruptly from patients who have been taking it in moderately high doses over a long period of time.

Scientists at the Addiction Research Center have been working on a variety of other drugs as well in their studies of addiction and in their search for non-addicting analgesics.

ALCOHOL AND BARBITURATE ADDICTION

In one series of experiments, substitution of alcohol for barbiturates has shed light on the theory of physiological addiction and has, in addition, provided some guidelines for the therapy of chronic alcoholics.

To test the theory that chronic intoxication with barbiturates may be equivalent pathophysiologically to chronic intoxication with alcohol, the investigators substituted alcohol for barbiturates in a group of ten addicts who had been continuously intoxicated with pentobarbital or secobarbital for 22 to 44 days. While the EEG pattern of the patients changed, the clinical symptoms of the two intoxications were similar. The usual symptoms of withdrawal from barbiturate intoxication were significantly decreased during alcohol intoxication. Although the subjects were kept intoxicated with alcohol for only 14 days, when the alcohol intoxication ended, an abstinence syndrome developed which was comparable to that observed in patients experimentally intoxicated with alcohol for between 48 and 87 days. The researchers conclude that alcohol is a partial, but not complete substitute for barbiturates in chronic pentobarbital or secobarbital intoxication.

The immediate value of the work lies in the treatment of chronic

alcoholics. Assuming that the substitution can work equally well in barbiturate treatment of alcoholism, when clinicians administer barbiturates, they are correcting a deficiency of alcohol by partially specific substitution therapy. A gradual reduction in dosage of sedatives should make possible a gradual rather than a precipitous loss of physical dependence on alcohol, thus preventing the appearance of convulsions and delirium.

STUDIES OF NORMORPHINE

Normorphine, a demethylated derivative of morphine, is being studied preparatory to possible use as an only slightly addictive but highly potent sedative and analgesic drug.

Normorphine produces morphine-like behavioral effects in man. These effects, however, appear slowly, are accumulated on repeated dosage of the drug, and disappear slowly. Given in enough repeated doses, the sedative effects of normorphine are more potent than those of morphine. In single doses, normorphine is less potent than morphine.

Tolerance to the sedative effects of normorphine develops more slowly than tolerance to the sedative effects of morphine, and abstinence symptoms following withdrawal from the drug after direct addiction are much milder than the abstinence symptoms after withdrawal from morphine.

The cumulative nature of the normorphine effect means that effects comparable to those of high doses of morphine can be achieved with relatively low doses of normorphine.

The addiction liability of normorphine is greater than that of codeine but less than that of morphine. Physical dependence upon normorphine is the mildest the Lexington investigators have ever observed in a morphine-series drug which is so potent in producing sedation.

Further study must be made of normorphine's analgesic potency and possible side reactions before its clinical role can be fully determined.

EFFECTS OF NALORPHINE

Another morphine-related drug, nalorphine (N-Allyl-normorphine), has been under scrutiny by Addiction Research Center scientists. Nalorphine, a non-addicting drug, acts differently depending upon whether it is administered alone, or following one, several, or an addicting number of doses of morphine. Under some circumstances it acts as a strong

morphine antagonist, while under others it produces mild to moderate dizziness, lethargy, and other symptoms. Most important, nalorphine counteracts the respiratory depression which develops as a result of accidental opiate poisoning, or during the analgesic administration of opiates.

Mothers who have received opiate-like drugs during the course of labor frequently show respiratory depression. Occasionally the newborn infants of these mothers fail to gasp or cry. Nalorphine, administered before the second stage of labor, acts to prevent this respiratory depression in both mother and child.

Nalorphine can also be used in the diagnosis of opiate addiction. Under proper medical and legal supervision, nalorphine is administered to suspected addicts. Abstinence symptoms (perspiration, dilated pupils of the eye, gooseflesh, nausea, vomiting) develop if the subject has been taking morphine or an equivalent opiate in sufficient doses to create physical dependence.

HUMAN INTERACTION

Not all the behavioral effects noted in experiments performed with drugs are attributable to the drugs themselves. Nor are these effects due to "spontaneous" activity of the nervous system. People interact with other people and with other aspects of their environment, and these interactions are responsible for a great part of our human behavior. Institute psychiatrists and social scientists have, during the past year, carried on a great number of studies of the relationship between social environment and behavior.

DRUGS AND TREATMENT SETTING — A group of NIMH grantees are studying the effects of tranquilizing drugs upon different types of patients in both custodial and therapeutic settings.

Four chronic schizophrenic patients were transferred from a closed ward to a small, intensive treatment center. Two of the patients had been "good" in that, although their behavior was regressed and deteriorated, they caused the staff little trouble. The other two had been problem patients--destructive and sometimes assaultive.

On tranquilizing drugs in the new setting, the two problem patients improved immediately, probably, the report states, because the therapeutic milieu was able to give them much more assistance and encouragement in satisfying simple needs, such as feeding and clothing themselves. The "good" patients became worse in the new setting, probably because they had adjusted in a relatively satisfactory manner to the routines of the custodial hospital.

This preliminary report emphasizes the need for study of the entire psychotherapeutic setting in which patients are receiving drugs if the effects of the drugs are to be understood.

ENVIRONMENT AND DRUG ADDICTION — Some of the social and environmental forces involved in drug addiction were underlined during the past year by Dr. John A. Clausen, Chief of the NIMH Laboratory of Socio-environmental Studies, in a review of current research on the subject. Narcotics use, according to Dr. Clausen's report, is highest in those population segments where income and education are lowest and where there is the greatest breakdown in supporting social structure--the family, the neighborhood, and so forth.

Induction to narcotics use is almost always a social process, and addiction itself leads to progressively greater estrangement from conventional social norms and groups. The associational pattern of the addict and his self-image tend to be organized around the maintenance of the addiction. The strong social stigmatization surrounding the addict further estranges him from conventional society.

On the basis of the variety of studies included in his review, Dr. Clausen concludes that it is unrealistic to assume that individual treatment methods alone can be effective in dealing with the problem of addiction. Addiction must be understood as a social phenomenon and its problems must be approached in social terms.

DEMONSTRATION CENTER AND DRUG ADDICTION

In line with this analysis of the problems of drug addicts, the Community Services Branch of the NIMH has set up a demonstration center in New York City to offer follow-up assistance for Lexington Hospital probationaries and parolees in New York. The aim of the center is to prevent relapses among cured drug addicts by helping them utilize the facilities of social agencies and by providing consultation to the agencies so that they will better be able to meet the needs of the former addicts. Operated in relationship with a number of social agencies in New York, the center has already begun preliminary tabulation of its findings. Early results show, in general, high rates of readdiction after discharge from the Lexington Hospital. Rates of readdiction, however, differ with age, sex, ethnic background, length of hospital stay, and other factors.

There is considerable evidence that the prevalence of addiction in the total population decreases with advancing age, but the reasons for this are unknown. It has often been suspected that at some stage in their maturation many addicts become abstinent. It is hoped that the continuation of this study over time will begin to shed light on when and how and why this takes place among different groups. Answers to these questions might be of great value in reducing over-all prevalence rates at all age levels.

Experience has shown that there are obstacles both in the patient and in the community that interfere with the full use of community services for the addict discharged from the hospital and in need of rehabilitation within the community. Since the project is demonstrating unequivocally that most addicts can be kept track of, its results will certainly provide a much deeper understanding of how community facilities can be more effectively used in the rehabilitation of former narcotics addicts.

SCHIZOPHRENIA AND THE STRUCTURE OF A CITY

The relationship between environmental factors and mental illness is being studied in a number of ways by NIMH scientists and grantees. Studies of rates of hospitalization for schizophrenia in very large cities have consistently shown that the rate is highest for the lower socio-economic levels and lowest for the highest levels. Somewhat smaller cities (in the size range of Milwaukee, Peoria, and Omaha) have shown somewhat lower correlations between hospitalization rate and socio-economic level.

In a new study which Drs. John A. Clausen and Melvin L. Kohn of the Laboratory of Socio-environmental Studies of the NIMH have carried on in Hagerstown, Md., a small city (pop. 36,000), no discernible correlation between socio-economic status and rate of hospitalization for schizophrenia has been found.

The most likely interpretation of this greatly decreased correlation in smaller cities is that there may be a direct relationship between the size of the city and the degree to which rates of schizophrenia correlate with such indices of socio-economic status as area of residence and occupational level. The social phenomena indexed by occupation and area of residence in Hagerstown may be quite different from those indexed by the same items in, say, Chicago.

The negative, but very definite findings of this study pose a new question for investigation: What are the values, attitudes, behaviors, and relationships affected by the socio-economic structures of cities of various size which are most significantly related to the development of schizophrenia?

CULTURAL DETERMINANTS IN SCHIZOPHRENIA

Other studies of schizophrenia focus cultural background as a determinant of the form the illness takes. NIMH grantee Dr. Marvin Opler, with Dr. Jerome L. Singer, has reported that a group of schizophrenic patients who grew up in Irish families differed markedly from a group raised in Italian families, and that neither group fitted neatly into traditional schizophrenic categories. Thirty Irish and thirty Italian patients, matched almost exactly for age, education, IQ, length of hospitalization, economic level, marital status, and recency of family's immigration to this country, were examined intensively in many ways: through study of family background, medical and psychiatric history, and hospital behavior record; by personal interviews; and by means of a battery of 13 standard

psychological tests. In addition, field surveys had previously been made of Irish and Italian family life in the section of New York City from which the patients came, in an attempt to establish clear and consistent patterns of organization in both ethnic groups.

On the basis of studies of family life, the investigators attempted to predict the symptom patterns of the patients in advance of detailed personal examinations. The typical Irish family, the investigators found, tended to have a dominating mother, a rather weak father, sin-tinged feelings about sex, and the general attitude that the active expression of emotion is undesirable. By and large, the Italian families were dominated by the fathers, encouraged the free expression of emotions and passion, and attached little or no sin or guilt to sex. From these attitudes, the investigators predicted that the Irish schizophrenics would have strong feelings of guilt about sex, would have the homosexual inclinations common in schizophrenics, but would suppress them and keep them latent, would show strong anxiety and fearfulness, would manifest little active hostility, would substitute delusions and fantasy for overt action, and would show a high incidence of alcoholism. By contrast, it was predicted that the Italian schizophrenics would have few guilt feelings about sex, would tend to have a history of active homosexuality, would be openly hostile, would have histories of violent behavior and defiance of authority, would have few if any fantasies and delusions, and would have a very low incidence of alcoholism.

Examination of the patients revealed that the predictions were highly accurate. Interestingly, the one Italian whose symptoms and attitudes departed at all from the norm of the other 29 Italian patients was the only one who had a different cultural background, coming from the north of Italy while all the others came from southern Italy and Sicily.

The investigators conclude that each of the patterns of schizophrenia studied bears the strong imprint of underlying family experience and patterns of stress. Different cultures, it appears, not only enforce different standards for normal behavior, but, because of the stresses and frustrations peculiar to each of them, also foster different patterns of maladjustment and mental disorder.

REHABILITATION AND THE PATIENT'S FAMILY

Interpersonal and social factors are as important in the rehabilitation of the mental patient as they were in determining the form his illness took in the first place. One investigator feels that rehabilitation could be more

successfully carried out if mental health personnel were more aware of the beliefs and values of the patient's family.

Charlotte Green Schwartz, as a member of the staff of the Laboratory of Socio-environmental Studies, NIMH, completed studies which have now been published in a paper "Perspectives on Deviance: Wives' Definitions of Their Husbands' Mental Illness," in which she examines the variety of ways in which wives of mental patients view and define their husbands' illness. She finds, for example, that a wife may place little emphasis on her husband's strange ideas if he can fulfill his role of wage-earner, husband, and father. The psychiatrist on the other hand realizes that these "strange ideas" may be delusions and evidence of severe pathology even though the patient functions in other areas. A wife whose husband becomes verbally hostile in contrast to his passive pre-hospital behavior, may think he is getting worse, whereas the same behavior to the psychiatrist might mean that the patient is improving. These attitudes, divergent as they are from accepted psychiatric views, must be taken into account in planning rehabilitation of mental patients, as must the wives' expectations of the returning mental patients.

The author concludes from her survey that many of the present difficulties in helping families reintegrate patients into community life lies in the fact that psychiatric personnel tend to approach the problem as if there were one answer or one set of principles by which all families can be guided in their relationships with patients. She urges that rehabilitation workers find out the unique ways of thinking and of relating to each other that constitute the life of a variety of subgroups from which patients come to the mental hospital, and use this information in planning the rehabilitation of the mental patient.

CULTURAL VALUES AND MENTAL RETARDATION

Cultural and social values even play an important role in the diagnosis of mental retardation in our society. In a survey of research on retardation carried on by the National Association for Retarded Children under a joint grant from the National Institute of Mental Health and the National Institute of Neurological Diseases and Blindness, Drs. Seymour Sarason and Thomas Gladwin have found that different societies and various subcultures within our own society have different values and make different demands upon their members. Hence, a person classified as retarded in a group holding one set of values will be an able and functioning member of a group with a different set of values.

Our society, by and large, tests for retardation in terms of school performance and academic achievement. Subcultures which do not attach our normally accepted high values to schooling tend, therefore, to produce a greater number of children likely to be classified as "retarded" in school.

Evidence that our criteria for diagnosing retardation are limited and biased by our cultural values is the fact that statistics show a much greater proportion of retarded in the population under 16 years of age than in the population over 16. The authors infer from these figures that many of the school age "retarded" manage to function competently and to support themselves in our society after they leave school. The authors recommend, in view of these findings, that new approaches to the theory and measurement of intellectual functions be made, since existing tests measure, at best, a very narrow range of these functions.

NEW TRENDS AND DEVELOPMENTS

A number of program developments during the past year will contribute materially to the implementation of research findings in the mental health field both by increasing the number of trained specialists in basic research and clinical work, and by improving programs of care for the mentally ill and experimenting with new techniques of care and treatment.

ADDITIONS TO TRAINING PROGRAM

Three significant additions have been made to the Training Program of the National Institute of Mental Health. One of these is the Senior Stipend Program, designed to meet the need for advanced training in mental health on an interdisciplinary or cross-disciplinary basis. Stipends are available for a limited number of individuals of outstanding competence on a post graduate level who, with advanced training, could use their basic skills to make a more effective contribution to an understanding of mental health and mental disorder.

Another addition is the program of research training in the basic sciences. With the growth of interest in the application of basic disciplines to mental health research, the Institute decided to use some 1957 funds to award teaching grants and traineeships for pre- and post-doctoral studies in chemistry, physiology, pharmacology, and the social sciences, so that students in these fields will have an opportunity to use their training for mental health research. As an example of the effectiveness of this program, it is estimated that in one university, support under this program will yield from 15 to 20 social scientists for work in mental health over a seven-year period.

The third addition to the Training Program of the Institute is one of training grants for doctoral programs oriented to the training of research personnel in such traditional areas of psychology as personality, child, physiological, social, and experimental psychology, and in programs organized about specific problem areas, presently characterized by acute professional shortages, such as mental retardation, geriatrics, delinquency, alcoholism, psychopharmacology, school and community mental health. This addition was dictated by the need for research personnel not only in the clinical field, but also in those areas which involve the study of a wide range of human behavior.

These developments, together with the existing broad training program, help the Institute more fully fulfill its responsibilities to assist in the improvement and development of mental health training and to train greater numbers of people equipped to serve in this field.

MENTAL HEALTH PROJECT GRANTS

Another significant development in the Institute's program has been the implementation of Public Law 911, the Health Amendments Act of 1956. Title V of this Act authorizes the Surgeon General, upon recommendation of the National Advisory Mental Health Council, to award grants for special mental health projects. The aim of these project grants is to support the development of improved methods relating to care, treatment, and rehabilitation of the mentally ill, including the development and establishment of improved methods of operation and administration of State Mental institutions.

On July 1, 1957, two million dollars was available for support of these grants. The National Institute of Mental Health has reviewed 137 applications for grants from 34 States and three Territories. Of this number, 44 have been awarded grants by the Surgeon General on recommendation of the Advisory Council, for a total of $1,385,306. In case of many of the rejected applications, consultation was provided and a visit to the applying individual or agency was made to keep the idea alive, if it was a good one, and to encourage resubmission of an improved application. Recipients of grants include State Departments of Health, State Hospitals, State Institutions for the Mentally Retarded, private hospitals, Community Mental Health Clinics, universities, residential treatment centers for children, rehabilitation centers, and such professional organizations as the National League for Nursing. Examples of the sort of program supported are a project to develop ways and means to sustain the geriatric patient extra-murally and cut down, if possible, the admission of non-psychotic geriatric patients to the public mental hospital, a study of the role of practical nurses as a possible solution to the problem of the shortage of nurses in State Hospitals, and demonstration of a psychiatric rehabilitative service for young inmates of a county jail.

INCREASED HELP FOR COMMUNITY MENTAL HEALTH

Another significant trend in the strengthening of community mental health service is the adoption by four more States -- California, Minnesota, New Jersey, and Vermont -- of legislation providing for State grants-in-aid to localities for community mental health services.

This type of legislation is highly significant in its impact on the future development of community mental health programs. At the local level, the availability of State matching funds makes it possible for more communities to initiate new programs of community mental health services. Where communities are already completely supporting mental health services, State matching funds release local funds which can be used to expand and improve existing services.

At the State level, the legislation indicates the acceptance by the State of responsibility for helping to finance local mental health services on a continuing basis. State appropriations for community mental health may be expected to increase. An eventual development of local services throughout the State is implied. Also State funds act as a binder in bringing closer working relationships of State and local mental health staffs.

The use of Federal grant-in-aid funds is also affected. Part of Federal grants are now being used to initiate mental health clinics in communities. With the availability of State funds for this purpose, Federal funds can be used increasingly for demonstrations of new types of services, pilot projects, training and research.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

NEUROLOGICAL AND SENSORY DISORDERS

1957

Items of Interest on Program Developments and Research Studies Conducted and Supported by the National Institute of Neurological Diseases and Blindness

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The year 1957 has seen a continued development and expansion of the neurological and sensory research program. The trend toward broad-scale cooperative programs--programs tying many research institutions and disciplines into an organized quest of a series of common objectives--continued during the year. However, this trend was complementary to research efforts directed toward single disease problems and those carried out by individual investigators or institutions.

The year saw continued progress along both clinical and basic research lines, with important findings being made in both areas. These will be discussed in detail in the following pages. However, some of the pertinent research developments may be summarized as follows: (1) diagnostic advances relating to eye disorders; (2) major progress in the treatment of temporal lobe epilepsy through surgery; (3) significant biochemical findings bearing on multiple sclerosis; (4) continued progress in cerebral palsy and Parkinsonism; (5) basic research advances relating to the regeneration of nerve tissue; (6) improvement of a brain tumor detection device and utilization of a technique which is shedding much light on the fine structure and function of the central nervous system; and (7) advances in knowledge of the structure, function, and chemistry of the brain.

The training of badly needed scientists for research and teaching in the neurological, ophthalmological and related disciplines continued to expand and to net concrete results. During fiscal 1957, about 60 neurologists and 90 ophthalmologists completed their training under the Institute's programs. An estimated 545 scientists have entered training under those programs during the year.

The Institute also developed a new specialized program under which grants for advanced training previously only available to the clinical disciplines also became available for study in the basic sciences. The program is specifically concerned with encouraging advanced training in such basic science areas as neurochemistry, neuropharmacology, neurophysiology, and neuroanatomy--areas in which personnel are in great demand. Persons eligible for this new program must have completed residency requirements in a clinical specialty or its equivalent or must have had at least three years of postdoctoral training or research experience. The program is fully consistent with the premise that basic research underlies virtually all clinical progress.

I. BASIC RESEARCH DEVELOPMENTS

Basic research lies somewhere behind virtually every effective preventive, treatment, or cure developed in the modern era. Without basic research, for example, the polio vaccine could not have been developed nor could retrolental fibroplasia, the blinding disease which strikes so many premature infants, have been eliminated as a major disorder. Thus, it is fully understandable that the Institute during 1957 continued to emphasize basic research both at Bethesda and through its research grant program. Further, that emphasis was fully in accord with developments in the expanding clinical research program. In this connection, it should be noted that the bond between clinical and basic research was nowhere more evident than in the concentrated research upon the structure, function, and anatomy of the human and animal brain. Such research was conducted both at the Institute and through grants to cooperating investigators.

PROGRESS MADE IN NERVE TISSUE REGENERATION

The Institute has been conducting animal experiments with a view to bringing about the regeneration of destroyed nerve fibers in the central nervous system. For example, if the spinal cord is severed, it has for many years been considered that nerve fibers never could grow back across this cut. Within the past few years, however, it was observed that their failure to grow back was due to thick scar tissue, originating on the surface of the spinal cord, and growing down into the cut.

By putting a porous collar around the cut ends, researchers at Columbia-Presbyterian Medical Center in New York found they could prevent the ingrowth of blocking scar tissues. Nerve

fibers thus find a clear path for regrowth. These scientists succeeded in regenerating nerve fibers across a 2.5 cm. gap in the spinal cord of cats.

NEW STAINING TECHNIQUE SHEDS LIGHT ON NEURAL COMMUNICATION

The synapse is that point or "space" in the central nervous system in which the impulse passes from one nerve cell to another. Many neurological difficulties occur when communication of the impulse fails or is impeded at the synapse. The past year has revealed much about what goes on in the synapse.

One development which has helped to shed considerable light on the processes at work in the synapse is a new silver staining technique developed by an Institute investigator and--during the past year--used in many animal experiments. Before this technique had been developed and applied, it had been impossible to see all or even most of the terminals (boutons terminaux) of the nerve cell structures feeding into a given synapse. With this technique--used in conjunction with the electron microscope--it is possible to see all or virtually all these terminal endings at the same time. Thus, it is possible to trace all synaptic connections relating to a single nerve cell.

The key to the success of the technique is that it selectively stains the nerve cell endings as opposed to other structures in the synaptic area. A special mordant, a substance which fixes or hardens the silver stain, makes this precise selectivity possible. The new staining technique, then, is a basic research finding of the greatest importance. Through its use in the study of animal nervous system, it is expected to reveal much about nerve impulse transmission.

PROGRESS IN STUDY OF BRAIN INFLAMMATION

Study is revealing much about the changes in the central nervous system brought on by inflammation of the brain. It is through this inflammation that actual brain damage may occur in encephalitis, and on occasion in such common diseases as measles and mumps.

A useful experimental tool has been the production in animals of a picture resembling the human disease. This is produced when an animal is sensitized to brain tissue and is spoken of as an allergic encephalomyelitis.

The Institute, in October, 1957, sponsored a symposium on Experimental Encephalomyelitis as it related to other diseases of man and animals. The conference was attended by more than sixty prominent research scientists from research centers throughout the nation. The conference members discussed the specific causes of allergic encephalomyelitis and the nature of the inflammatory reaction of the brain.

NEW DATA REDUCTION SYSTEM GREAT TIME-SAVER IN BIOPHYSICAL RESEARCH

The past year has seen the development of a new electronic system for rapidly simplifying and reducing raw experimental data to tabular or graphic form. The system consists of three component parts: a reader, an electric typewriter, and an electromechanical plotter. It does not compute data but allows for rapid input and output of data for computers.

The system makes possible a considerable increase in the amount of data that can be collated and evaluated in a given period of time. It greatly facilitates the reading, tabulating, and plotting of raw data and allows for greater flexibility in its presentation for computation.

The electronic system is being used by Institute biophysicists for the processing of data relative to the electrical properties of nerve impulses in animals. The data reduction system also is used in conjunction with commercially available computing equipment.

II. COLLABORATIVE AND COOPERATIVE PROGRAMS

On November 1, 1956, the Institute announced the launching of the first of several major field investigations, that is, broad-scale cooperative or collaborative programs. Three such investigations are well under way and two others are in the advanced planning stages. In this context, a collaborative study is one involving a number of research centers in which the Institute serves as a focal, coordinating influence. A cooperative study is one in which an outside research center coordinates the program with the Institute playing no direct role in the investigation.

A. The Perinatal Period Study--Cerebral Palsy, Mental Retardation, etc.

A key collaborative study in process is concerned with brain damage occurring during the perinatal period, which covers pregnancy and about one month after the birth of a baby. The long-term aim of the study, which may continue for ten years or more, is prevention--prevention of cerebral palsy, mental retardation, epilepsy, and various forms of congenital blindness and deafness.

The collaborating institutions are evaluating a variety of factors, some implicated, and some suspected of causing perinatal damage. These include lack of oxygen, blood incompatibility between mother and child (the Rh factor), prematurity, and infections occurring during pregnancy. Genetic factors, though believed to play a relatively minor role in cerebral palsy and mental retardation, are also being evaluated. Specific knowledge as to exactly when and how brain damage is caused is essential in the effort to achieve the eventual prevention of cerebral palsy, mental retardation, and related disorders.

It is expected that the study will bring about 8,000 cases under study annually. Whenever possible, the mother-to-be will be studied through the entire period of pregnancy. Infants will be closely observed and tested for signs of neurological stress, and those showing signs of such stress will be closely followed for a number of months after delivery. Every effort will be made to correlate neurological signs with occurrences during pregnancy or at delivery which may have led to them.

Eventually, it is anticipated that fifteen institutions will collaborate with the Institute in the investigation. As of January 1, 1958, there were thirteen such institutions. These are: Boston Lying-In Hospital, Boston; Brown University, Providence; Charity Hospital, New Orleans, Louisiana; Children's Hospital, Philadelphia; Children's Hospital, San Francisco; Columbia University, New York City; Johns Hopkins University, Baltimore; Medical College of Virginia, Richmond; New York Medical College, New York; University of Minnesota, Minneapolis; University of Oregon Medical School, Portland; University of Pennsylvania, Philadelphia; and Yale University, New Haven.

FEASIBILITY OF PUERTO RICO MONKEY COLONY ESTABLISHED During late 1956 and early 1957, the Institute, working in conjunction with the University of Puerto Rico, acquired and modernized a monkey colony on an island in Puerto Rico along with various laboratory facilities. The colony and laboratory were set up with a view to playing a key role in the broad-based perinatal period study. They have already demonstrated their usefulness as a basic tool for the study of the entire process of gestation.

As of 1957's end, the Institute was able to report that the feasibility of breeding monkeys in caged colonies has been clearly established. As of the period in question, 70 timed

pregnancies were under observation. And, for the first time in primates, investigators were able to develop specific evidence of structural changes in the brain due to damage induced by lack of oxygen (anoxia).

Institute investigators now feel that the evidence is conclusive that lack of oxygen, when induced near the end of the gestation period, can bring on irreversible damage in the primate brain. This had been demonstrated previously only in smaller animals. The significance of this development insofar as brain-damaging disorders in humans are concerned is considerable inasmuch as the monkey brain closely approximates that of man in many respects.

B. Cooperative Programs in the Cerebral Vascular Field

In April, 1957, the National Institute of Neurological Diseases and Blindness launched the nation's first cooperative research attack against cerebral vascular diseases. These diseases, collectively known to the layman as "stroke", constitute the nation's third-ranking killer. It has been estimated that as many as 1,800,000 living Americans have suffered cerebral (brain) strokes and that deaths due to such strokes total about 175,000 annually.

The cooperative study was initially made possible by Institute grants to 17 medical research centers, and a number of additional centers are expected to join the study during 1958. The University of Iowa, Iowa City, is coordinating the investigation.

This cooperative investigation is making possible the study of thousands of patients who either have suffered a stroke or who show clinical signs indicating that a stroke might be coming on. The program is specifically concerned with patients suffering from cerebral vascular disease involving hemorrhage, blood-clots, blood tumors (aneurysms) and malformations of the arteries or veins of the brain. The study is expected to continue for 5 or 6 years.

Research results are expected to shed new light on the nature and causes of strokes and to open the way to more effective treatment methods. Relatively few data are now available on the effectiveness of the various methods now in use. Another aim of the cooperative program is to make possible the more accurate selection of stroke patients most likely to benefit from surgical or non-surgical therapies.

A cerebral vascular cooperative study, companion to that launched in April, is specifically concerned with evaluating the effectiveness of anticoagulant drugs in preventing strokes after initial signs have been recognized. This more recently-launched study (November, 1957), unlike the April program, is not concerned with treatment but only with preventives. It involves six research centers.

This more recent study is expected to provide a thorough evaluation of anticoagulant therapy, within 3 years.

An estimated 1800 patients will participate--a total far beyond the number available for study to any one institution except over a period of many years. Participating research centers are: University of Miami Medical School, Miami, Florida; Emory University School of Medicine, Atlanta, Georgia; Massachusetts General Hospital, Boston, Massachusetts; Duke University School of Medicine, Durham, North Carolina; University of Pennsylvania Hospital, Philadelphia; and Cornell University Medical School, New York.

Though the Institute's research grant program in the cerebral vascular field centers on the above cooperative studies, it should be noted that Institute grants also are supporting a number of specific research projects dealing with cerebral vascular diseases and conducted by individual hospitals and investigative teams.

C. Collaborative Studies Involving Viruses

Other broad-scale, collaborative studies launched during 1957 included one concerned with the effect of Asian flu on fetal development and another concerning the effects of insect-borne diseases upon the central nervous system. In both cases, the groundwork was laid and detailed plans discussed at meetings at Bethesda.

Insofar as the "flu" study is concerned, almost nothing is known of the afteraffects on fetal development. In light of the Asian flu epidemic which struck during 1957 and the possibility that there may be subsequent "waves," an understanding of these afteraffects is of the utmost importance. Those who attended the planning meeting at Bethesda pointed out the urgency of starting the study immediately, before mass vaccination influenced the epidemic characteristics and the interpretation of the individual's exposure to infection.

As matters stood at the year's end, at least ten medical centers were expected to join the Institute in a collaborative approach to the problem. It is expected that some 10,000 patients will come under study.

The value of the type of study contemplated has been underscored by the finding that the virus of German measles in the pregnant mother is capable of producing damage to the nervous system of the infant. The characteristics of the Asian flu epidemic have been such as to provide a unique opportunity to find out whether the flu virus is also capable of producing fetal injury, because serological specimens obtained from pregnant women during and after the epidemic make it possible to determine whether they actually have been infected by the virus. Hence, follow-up studies of the infants involved would provide evidence as to whether such infection does lead to defects in the offspring.

The study of insect-borne diseases, currently under development, is primarily concerned with the effects on the brain and nervous system of the encephalitides. These include the Western, St. Louis, Eastern, and Japanese types. It should be noted that these disorders are related to the occasional aftereffects of such common infections of childhood as measles and mumps.

The study is specifically concerned with determining how the encephalitides bring on neurological defects shown by disturbed behavior and inferior mental capabilities.

D. The Kuru Study

Kuru, which means "The Shakes," is a disorder which was recently discovered among the natives of a section of New Guinea. The disorder occurs among a population which does not exceed 15,000 but, among these people, about 1 percent of the total are affected. In certain parts of the area concerned, Kuru is responsible for about 50 percent of all deaths.

Although the total number of individuals affected is relatively small by our standards, Kuru does bear a striking similarity to a number of widespread degenerative diseases of the nervous system. And this is among the reasons for the Institute's active participation in a field investigation designed to determine the nature, course, and causes of the disorder. Pathological specimens from New Guinea were under careful study in the Institute's laboratories as of the end of 1957.

II. PROGRESS IN INDIVIDUAL DISEASE CATEGORIES

Clinical progress during the past year, like that in basic research, has been substantial in many areas. Following is a resume of clinical and related developments concerning the various kinds of neurological and sensory disorders with which the Institute has been concerned.

CEREBRAL PALSY SYMPTOMS RELATED TO SPECIFIC AREAS OF BRAIN DAMAGE

Though the Institute has emphasized prevention in the cerebral palsy field, the year also has seen important advances in other areas. A fruitful study reported on during the year was one conducted by Dr. Malcolm B. Carpenter, an Institute grantee at the College of Physicians and Surgeons at Columbia University.

Dr. Carpenter was able to relate ataxia (muscular incoordination), tremor, and dyskinesia (impairment of power of voluntary motion) to lesions in specific areas of the monkey brain. The lesions were induced in various parts of the cerebellum, the segment of the brain primarily concerned with coordination of the muscles and maintenance of body equilibrium. Some 75 monkeys were used in the study.

All the symptoms which Dr. Carpenter was able to relate to specific areas of brain damage are characteristic of cerebral palsy in man. Thus, the specificity of the relationship which the investigator was able to establish sheds much light on the site and nature of damage in man afflicted by cerebral palsy inasmuch as the monkey brain is similar to that of man in many respects.

In order to establish the precise, specific relationships between brain damage and cerebral palsy symptoms, Dr. Carpenter made use of the motion picture camera as well as of written records of animal responses. Thus motion pictures were taken of noteworthy physiological disturbances so that comparisons could more readily be made between physiological activity in the same animal at different times and between different animals.

EPILEPSY

SURGERY HIGHLY SUCCESSFUL IN TEMPORAL LOBE EPILEPSY

The Institute--at the end of 1957--made a five-year assessment of its surgical therapy program in temporal lobe or psychomotor epilepsy. There are many different types of epilepsy but it is estimated that almost 40 percent of the total fall into the temporal lobe category.

In any event, the Institute is able to report that 85 percent of all temporal lobe epilepsy cases coming to surgery during the period in question have been found to be either completely free of seizures after the surgery or to have had the number of seizures drastically reduced.

Specifically, fifty-five percent of the epileptics coming to surgery had not more than one seizure in the post-operative period. Thirty percent had three or fewer seizures following surgical treatment. Only fifteen percent had as many attacks after surgery as before. Of these, a considerable number had epileptic involvements both in the temporal lobe and elsewhere.

DRUG-INDUCED SEIZURE FOUND TO BE OF DIAGNOSTIC VALUE

Epileptic seizures induced by the drug, metrazol, have been found to be of considerable diagnostic value. Seizures so induced are very similar to the patient's habitual attack. Thus, Institute scientists are able to study what amounts to the patient's "normal" seizure pattern under the best possible conditions of observation. Diagnosis is thus not only more precise but much data not heretofore available have been gathered relative to the initial signs of the attack as well as to the manner in which the attack develops during its course.

MULTIPLE SCLEROSIS

Though medical science is still seeking evidence of the specific cause or causes of multiple sclerosis as well as effective therapy, considerable progress was made during 1957 in the research attack against the disorder.

NEW TECHNIQUE REVEALS CHANGES IN SPINAL FLUID OF SCLEROSIS PATIENTS

A research team working under the leadership of Dr. Francis M. Forster at the Georgetown University Medical School has developed a new technique for the measurement of constituents of the cerebrospinal fluid and has applied it clinically in the cases of persons with multiple sclerosis, brain tumors, and other neurological disorders. The work was conducted under an Institute grant.

The new measurement technique and its results are of importance both from the diagnostic and etiological points of view. Using it, the researchers found that the total protein-bound carbohydrates are significantly increased in the spinal fluid of patients with brain tumors but that the carbohydrates associated with gamma globulin were elevated in the fluid of multiple sclerosis patients.

BIOCHEMICAL DEVELOPMENT MAY OPEN WAY TO REVERSAL OF DEMYELINATION

A research development which may open the way to reversal of the process of demyelination was reported for the first time by an Institute investigator. Myelin is the "sheath" which covers the nerves of the central nervous system under normal conditions. In multiple sclerosis and related disorders, this substance deteriorates and fails to regenerate. This destructive process is known as demyelination.

The specific Institute achievement which may eventually make it possible to reverse the process involves the compound known as sphingosine. This is a fatty-amino-alcohol compound which is an essential element in the myelin sheath. More specifically, it is part of the lipids or fats which are found in the sheath. Sphingosine and the lipids of which it is a part tend to disappear during the process of demyelination.

The Institute now has discovered and characterized the specific manner in which sphingosine is synthesized in the body and has succeeded in synthesizing this vital compound in the laboratory by biochemical means. The investigator who has done so believes that the key to reversal of the demyelination process in multiple sclerosis may lie, in part, in a mechanism whereby the disappearance of sphingosine and the related lipids is reversed. In the year ahead, he plans to concentrate his efforts in the quest for a means of bringing about such reversal.

REPORT NEW DATA ON PREVALENCE AND MORTALITY IN MULTIPLE SCLEROSIS

Epidemiological studies sponsored by the Institute and by other organizations as well have revealed new data on both the prevalence and mortality rate of multiple sclerosis. Preliminary statistics indicate that the disorder is more common in the northern part of the United States than in the South and much more common in western Europe than in various Far Eastern countries. It is almost non-existent in Japan.

The highest death rates from multiple sclerosis--according to a World Health Organization survey--in 13 European and 7 non-European countries studied were noted in Scotland, France, and Northern Ireland. The lowest rate, understandably, was in Japan. Generally speaking, it was found that the number of females dying of multiple sclerosis was somewhat higher than the number of males.

Institute epidemiological studies are continuing in conjunction with several other organizations. If more comprehensive statistics demonstrate a clear-cut relationship between geographical area and multiple sclerosis prevalence, an important clue as to the cause of the disorder will undoubtedly have been discovered.

MENTAL RETARDATION

The long-range collaborative research program directed to development of techniques for preventing the onset of mental retardation, cerebral palsy, and allied disorders has been discussed in detail on previous pages. This program--vastly important though it is--is by no means the only major development in the mental retardation field during the past year.

COMPREHENSIVE SURVEY OF MENTAL RETARDATION RESEARCH COMPLETED

During 1956, Dr. Richard L. Masland, head of the Neurology Department at the Bowman-Gray Medical School in North Carolina, and associates in the mental health field were awarded a grant to make a comprehensive survey of mental retardation research in this country and in various areas abroad. The survey was jointly sponsored by the National Institute of Neurological Diseases and Blindness, the National Institute of Mental Health, and the National Association for Retarded Children. Dr. Masland concerned himself with an exhaustive study of mental retardation research from the neurological point of view.

During the past year, the survey was completed and two reports--one prepared by Dr. Masland and reflecting his findings in the neurological area and the other prepared by his associates and dealing with mental health aspects of retardation--were completed. Both reports were scheduled for publication in January 1958. Comment on the second report--that dealing with the mental health aspects--appears in the appropriate Mental Health Institute documents. This document will concern itself only with Dr. Masland's findings.

Dr. Masland's report is the first to have delved so comprehensively into the neurological aspects of mental retardation research. As such, it presents, in an organized fashion, the major factors which must be taken into consideration in pressing the attack against the neurological factors inherent in the mental retardation problem. In assessing existing research trends, research findings and research objectives, the report will be of incalculable value in plotting future research in a manner which will direct immediate attention to gap areas as well as avoid "blind alleys" which have already been exploited. More, the report will serve as a communications "bridge" for investigators in the mental retardation field so that each may be informed as to what the others are doing.

Detailed discussion of the report is beyond the scope of this "Highlights" document. However, it should be noted that Dr. Masland's findings strongly indicate that a biochemical attack against the metabolic defects typical of many mental retardation cases is among the most hopeful approaches of the immediate future. The author also makes a strong case for a specific relationship between the economic and social factors bearing on the pregnant mother and the development of mental retardation and related disorders in the infant. This relationship is among the areas under careful study in the Institute's collaborative perinatal period study.

REPORT THYROID HORMONE EFFECTIVE IN PREVENTING "CRETIN" RETARDATION

Investigators at the University of Michigan (Drs. William H. Beierwaltes and George Lowrey) report that mental and physical retardation resulting from cretinism can often be prevented through the use of a thyroid medication. Cretinism or congenital hypothyroidism is a disease afflicting children born with a defective thyroid gland which produces a limited amount of thyroid hormone or none at all.

The investigators studied 83 cretins. They point out that the thyroid hormone is vitally necessary to development of nerve insulation and that mental and physical retardation takes place through a "short circuit" of the nervous system when there is inadequate thyroid to provide the needed insulation. They further report that the administration of the thyroid hormone to the cretins under study caused the latter to attain a normal I. Q. and good development.

MUSCULAR DYSTROPHY AND NEUROMUSCULAR DISORDERS

Muscular dystrophy, myasthenia gravis, and related neuromuscular diseases--these are among the most difficult neurological disorders with which medical research has sought to cope. Muscular dystrophy, which strikes primarily at children, is characterized by a wasting of muscle tissue. In myasthenia gravis, there is an impeding or blockage of impulse communication between nerve and muscle. At present, there is no known cure for either dystrophy or myasthenia gravis.

The year 1957, however, has seen some important progress in the research attack against these disorders. New diagnostic advances have occurred and considerable basic research relating to the manner in which blockage occurs at the nerve-muscle juncture has been carried out. Comprehensive study and evaluation of the impact of various chemical compounds on both nerve-muscle transmission and on directly-stimulated muscle have been conducted.

NEW MUSCLE DISEASE ATLAS IS KEY DIAGNOSTIC TOOL

In 1957, Institute investigators prepared an Atlas of Muscle Pathology in Neuromuscular Diseases which has been widely acclaimed in domestic and foreign medical journals. The Atlas is perhaps the most comprehensive of its kind and is considered to be an important aid in the diagnosis of the neuromuscular disorders. It has been published in both England and the United States.

The Atlas is divided into two basic parts. The first defines in detail the histological (fine structure) reactions of muscle to disease. The second correlates these reactions or various combinations thereof with specific neuromuscular diseases. The book is thus designed to serve as an aid to both the clinical neurologist in diagnosing these diseases and to neurological investigators working in the neuromuscular research field.

The data appearing in the new Atlas is based upon the exhaustive study of 121 cases of muscle disease by four Institute investigators.

NEW MUSCLE DISEASE DISCOVERED IN JOINT INVESTIGATION

Working in conjunction with Walter Reed Medical Center, Institute investigators last year discovered what, in effect, is a "new" muscle disorder. They demonstrated that paramyotonia congenita is a distinct and separate clinical entity rather than a variant of another disorder known as Thomsen's Disease. The latter view had previously been held by many investigators.

Paramyotonia congenita is extremely rare--there having been prior scientific reports on only two families in the United States who had it. It is generally characterized by two types of symptoms. (1) muscular contraction and tension usually associated with cold temperatures; (2) weakness in certain muscles of the extremities: The second symptom may occur independently of the first. The muscles of the eyes, face, and hands are usually involved and there is generally difficulty in elevating the arms.

EXTREME COLD FOUND TO BLOCK NERVE IMPULSE TRANSMISSION IN MAMMALS

An Institute investigator has found that extreme cold blocks nerve impulse transmission in rats. The transmission was restored by rewarming the animal. The finding confirms previous experiments involving frogs.

The study revealed that nerve-muscle transmission in living rats is most efficient between 36° and 15° C. It becomes impeded below 15° C and is blocked at 5°C. In the frog experiments, the relative figures were 25° to 5°C for optimum transmission, impedance at 5°, and complete blockage at -1°.

The investigator believes that it may shortly be possible to apply his findings clinically. He is currently conducting studies with a view to relating these findings to the development and progress of nerve- and nerve-muscle disorders in mammals. This is possible immediately because a condition not unlike muscular dystrophy has been produced experimentally in rats.

ADVANCES IN NEURO-SURGERY

Results of brain surgery relating to temporal lobe epilepsy have been reported in previous pages. This section is concerned with advances bearing on the broad field of brain surgery. Among such advances during 1957 were those concerned with the employment of hypothermia, with anesthesia, and with surgical instrumentation.

PLAIN DEVICE TO LESSEN IMPACT OF BRAIN HYPOTHERMIA ON HEART

One of the most promising research endeavors of the past year at the Institute lies in the employment of hypothermia, that is, of cooling, in brain surgery. Hypothermia is desirable in such surgery because it tends to reduce bleeding, and to give the surgeon a clearer area in which to work. It recently has been applied successfully in brain surgery to eliminate aneurysms (local ballooning of a blood vessel wall).

STUDY DEMONSTRATES SAFETY OF POTENT NEW ANESTHETIC IF USED WITH PROPER SAFEGUARDS

Fluothane, a new potent, non-explosive anesthetic agent, has come into recent use in human surgery. However, there have been reports that its use may have an adverse effect on the human heart.

Institute scientists have completed a detailed study of the anesthetic, using dogs. They concluded that Fluothane could be used without fear of any adverse effect on the heart if utilized with "extreme caution and meticulous attention to the respiratory and cardio-vascular signs." They spelled out safeguards in specific terms.

In particular, they urged that a vaporizer made specifically for Fluothane be used. This vaporizer delivers an exact concentration of the gas as required.

Inasmuch as Fluothane is both potent and non-explosive, it should prove a major boon in surgery if used with the caution prescribed.

KEY ADVANCE MADE IN SURGICAL INSTRUMENTATION

During 1957, several new instruments were developed for employment in neurosurgery. One of these was a human stereotaxic device. This instrument aids the surgeon in cutting into skull in a manner designed to reach the target area with the greatest possible precision so that no damage is done to the surrounding healthy tissue. Brain surgery, delicate at best, requires such precision for optimum results.

The new device is zeroed into position by X-ray control. With the aid of this instrument, areas deep within the brain may be reached with only one calculation and only one opening into the skull. Further, the precision of the device is such that it may be lifted from its position over the skull and replaced in precisely the same position it occupied at first. The advantage to the surgeon to whom precision is all-important is clear-cut, and the hazards to the patient are accordingly reduced should removal of the instrument be required before completion of surgery.

PARKINSONISM

During 1957, the Institute continued its study of various substances found to bring on Parkinson-like symptoms in animals, with a view to learning more about the nature and course of Parkinson's disease as well as to evaluating treatment possibilities in the drug field. During this period, Institute grantees reported on the effectiveness of procyclidine hydrochloride (Kemadrin), a drug that had been under evaluation in Parkinsonism cases for several years.

PROCYCLIDINE HYDROCHLORIDE FOUND EFFECTIVE IN PARKINSON THERAPY

Drs. Adolfo Zier and Lewis J. Doshay, of Montefiore and Presbyterian Hospitals in New York City respectively, reported that procyclidine hydrochloride is active upon all the symptoms of Parkinson's disease. They used the compound in treating a series of 108 patients who had not responded to other standard drugs. Also treated with the drug were 8 other patients with symptoms related to Parkinsonism.

Of the entire series of 116 patients, 42 were maintained on procyclidine hydrochloride alone, whereas 74 were given the compound along with other standard anti-Parkinson drugs with which it readily combines. The investigators report that 62 of the 116 patients--more than half--responded favorably. Forty-six were unimproved whereas 10 patients claimed their symptoms were worse. The side-reactions, the investigators report, were generally milder than those produced by other anti-Parkinson drugs.

BRAIN TUMORS

SUBSTANTIAL IMPROVEMENT MADE IN BRAIN TUMOR DETECTION DEVICE During 1956, the Institute reported the development of a device for precisely detecting and defining the limits of brain tumors without opening the skull. The device, which involves the employment of a technique known as "collimation detection," places an important new diagnostic tool at the physician's disposal.

The collimation detection device utilizes the isotopic tracer method coupled with electronic scanning and recording equipment. The isotopic substance (initially zinc) is absorbed by tumorous tissue at a faster rate than normal tissue and thus emits radioactive rays with greater intensity. The scanning and recording devices "pick up" the rays and distinguish between them and those emitted by surrounding healthy tissue. This, in turn, makes possible the precise location and definition of tumorous growths.

The first model of the device, reported on in 1956, was at least 80 percent effective in precisely locating tumors (gliomas), many of them deeply seated in the brain. The present model, which has been greatly improved and utilized on large numbers of patients, is about 90 percent effective. The current model is being standardized with a view to making it generally available through commercial channels.

Institute investigators who developed the detection device state that its precision is at least equal to that of other known tumor detection techniques. Further, they report, it can be used without the discomfort the patient often encounters with the other techniques and in cases in which those techniques are not feasible. Its precision in detecting certain types of brain tumors is undoubtedly superior to that of the other methods.

THE BLINDING DISEASES

The Institute's research program in the ophthalmological field continued to expand during 1957. Important diagnostic and therapeutic advances were reported both at the Institute and from other research centers. Some of these are discussed below.

IMPORTANT GLAUCOMA FINDING: RIGIDITY OF EYE VARIES WITH OCULAR PRESSURE

Institute investigators made a finding of considerable consequence in the diagnosis of glaucoma, the major blinding disease which is particularly evident in the age group of 40 and over. They found that the rigidity of the eye varies with intraocular pressure rather than remaining static as previously believed. The finding was based upon comparative studies of the eye of the cat.

Inasmuch as the measurement of intraocular pressure (pressure on the eyeball) is the basis of glaucoma diagnosis by the widely-used tonography method, any measurement which did not take rigidity changes into consideration might well fail to make an accurate diagnosis. The investigators have therefore worked out a corrective technique which compensates for the rigidity changes and thus makes for sound diagnosis. It is expected that the technique will be applicable to patients relatively soon.

PROGRESS MADE IN DIAGNOSIS OF DIFFICULT RETINAL DISORDERS

Among the most difficult blinding diseases to diagnose are those which fall into the general category of retinal disorders--particularly those of a progressive deterioration nature. Institute scientists have now developed electroretinography and allied techniques to that level where it is possible to diagnose clinical disorders of the retina previously impossible or difficult to diagnose in the early stages.

REPORT THAT ARTHRITIS MAY BE DIAGNOSED EARLY THROUGH CAREFUL EYE EXAMINATION

Investigators at the University of California School of Medicine report that careful eye examination in many cases may reveal the onset of rheumatoid arthritis one to four years before the basic arthritic symptoms appear. They have discovered that uveitis, iritis,and other eye disorders often appeared in association with rheumatic diseases. This discovery will contribute to the early diagnosis of arthritis and will encourage early treatment before crippling occurs.

TREATMENT ADVANCES MADE IN UVEITIS, RETINAL DISORDER

In 1956, the Institute reported on the successful employment of several substances in the treatment of a form of the blinding disease uveitis, toxoplasmosis of the eye. It also reported on a new uveitis diagnostic test employing what is known as the "agar-diffusion technique." Progress in use of both the compounds in question and the diagnostic test is continuing. In addition, a specialized file system has been introduced at the Institute which permits careful analysis of the clinical course of all uveitis patients with particular emphasis on any relapses which may occur. Studies are also underway to evaluate the relationship between uveitis and thyroid function.

SPEECH AND HEARING DISORDERS

In 1956, the Institute reported the discovery and tracing of the oliva-cochlear bundle, the nerve pathway linking the cochlea of the inner ear to the brain. The discovery, which was made possible by a specialized staining technique developed by an Institute investigator, revolutionized the approach to hearing research. Hearing had hitherto been thought of as a one-way process, with the brain simply serving as a relay station for impulses converted from sound waves. After the discovery of this nerve bundle, further studies proved the two-way role of hearing. The brain was found to have an inhibitory effect which is carried to the ear along this nerve bundle.

OLIVA-COCHLEAR FINDING OPENS WAY TO NEW RESEARCH APPROACHES IN 1957

The discovery and tracing of the oliva-cochlear bundle had a major impact on the direction of Institute hearing research during 1957. Using the basic data revealed by the finding, investigators invoked conditioned reflex techniques employed by the psychologist to train animals to respond to various types of auditory stimuli. Then they recorded the electrical changes in brain and ear which accompanied such responses.

Further, specific changes in these responses resulting from disease or injury were carefully observed and evaluated through surgery on the animals. These observations and evaluations

are now being correlated with those made in the examination of patients whose brains have been harmed by injury, stroke, or tumor. The goal is to develop even more specific knowledge as to how injury and disease affect the hearing system.

STUDY REVEALS INNER EAR ORGANS OF FETUS ARE MORE RESISTANT TO DAMAGE THAN VARIOUS OTHER ORGANS

A Harvard Medical School study made by grantees Drs. Theodore H. Ingalls, George Kelemen, and Francis J. Curley reveals that inner ear organs of the mouse fetus resist damage when the pregnant mouse is exposed to lack of oxygen (hypoxia). This contrasts with findings that the brain, eyes, and other organs are damaged when exposed. The investigators attribute the hearing organ resistance to the fact that it is relatively lacking in blood supply and relatively independent, at least during the early fetal stages, of the development of the nervous system as a whole.

This finding bears on the Institute's overall investigation directed to determining the nature and causes of damage to the human brain brought on during the perinatal period. Such damage, as has already been pointed out, often brings on neurological disorders like cerebral palsy, mental retardation, and epilepsy.

HIGHLIGHTS OF PROGRESS

CLINICAL CENTER

1957

Much of today's knowledge of human physiology and of methods for diagnosing, treating, and preventing disease, is based upon observations and tests of people in normal health conducted over past decades and centuries. The medical advances of 10, 20 and 100 years hence will also depend to a great extent on the new knowledge being obtained now through study of individuals and groups enjoying normal health.

It is believed that most of the important chronic illnesses are due to internally originating malfunction of organs and processes rather than invasion from without. A considerable part of today's research aimed at better methods of treating and preventing these illnesses must begin with attempts at precise determination as to just where and how the dysfunction differs from normal. Therefore, knowledge of the complex chemistry of the human body in the normal state is needed in order to detect, measure and evaluate these abnormalities.

This kind of fundamental study--conducted both in ill and in well humans--must employ extremely precise and difficult laboratory technics in conjunction with medical, nursing and dietary care that can be provided best under especially equipped and staffed hospital conditions. The Clinical Center at the National Institutes of Health is one of the largest institutions in the world thus equipped and staffed.

Soon after the Center opened in July, 1953, arrangements were completed with two church organizations (Mennonites and Brethren) through which healthy, highly motivated young men and women could volunteer to participate as normal subjects in the NIH research programs. In four years 250 have so volunteered and served for periods ranging from a few weeks to more than two years. By January 1, 1958, their contribution had totalled approximately 22,650 man-days.

The volunteers are admitted as hospital patients and are under direct medical supervision of NIH physicians. Attention to their medical welfare is as painstaking as the attention accorded the sick study patients. Special policies and procedures have been developed to safeguard against their being subjected to undue

hazard, and to insure that their voluntary participation in various studies is based on full information as to its purpose, the methods to be used, and the tedium or physical discomfort involved.

The following are typical of the studies in which these volunteers participate:

Turnover of Plasma Iron:

Small amounts of radioactive iron are injected into the blood stream. The plasma iron concentration and the amount taken up by the red blood cells is determined from frequent blood samples taken over the following two weeks.

Mechanisms of Action of Orinase:

How this new oral treatment for diabetes achieves its effects is studied by giving gradually increasing amounts intravenously to normal volunteers over periods ranging from one-half to two hours. Complete blood, liver, and kidney studies are made before, during, and after the infusion.

Fate of Hydrocortisone in Man:

This steroid compound is injected intravenously. Blood samples and urine are then collected and subjected to chemical analysis. Similar studies are made with the volunteers simultaneously receiving doses of hormones over a period of 10 days or while being subjected to physiological stress.

One of the concepts underlying the Clinical Center is that its concentration together under one roof of carefully selected patients and practitioners of virtually every branch of laboratory and clinical science will expedite research on many difficult basic problems in medicine. There have been many examples of the validity of this concept. One of the most recent occurred in the research program of the National Institute of Arthritis and Metabolic Diseases and led to solution of a problem which has vexed scientists for many years.

Alcaptonuria is a comparatively rare metabolic disease often characterized in the later years of life by serious complications, including arthritis and arteriosclerosis. That the condition is hereditary and due to the absence of an enzyme in the liver was postulated 50 years ago. In order to prove this theory it was

necessary to obtain a small portion of the liver of an individual having the disease and subject it to difficult chemical analysis--a potentially hazardous procedure, not to be undertaken unless abdominal surgery was necessary for other reasons.

During early 1957 there were in the Clinical Center at the same time: Staff investigators especially interested in and actively working on the problem; an alcaptonuric patient who suddenly developed a bleeding ulcer of the esophagus which required surgical intervention; and a visiting English surgeon, world-renowned for his work on the esophagus. When the circumstances were fully explained to the patient, he readily agreed to permit a specimen of liver to be taken when the abdomen was entered for the repair of the ulcer. With special techniques developed in previous work with this disease, it was then possible to determine that the missing enzyme was homogentisic acid oxidase. A most important aspect of this finding is that the demonstrated causal relationship between a particular metabolic defect and a particular form of arthritis may lead to better understanding of other metabolic defects and other forms of arthritis.

HIGHLIGHTS

OF PROGRESS

IN BIOLOGICS CONTROL

1957

Items of Interest on Program Developments and Research Studies

Conducted by the Division of Biologics Standards

The primary function of the Division of Biologics Standards is the control of the safety, purity, and potency of biological products, implemented through a process of licensing. This function, of necessity, involves close cooperation with other official and non-official agencies and laboratories operating in related fields of interest. For example, the Department of Defense in relation to the military use of these products, the National Research Council, state and local laboratories, as well as the World Health Organization in cooperative studies and interchange of information on biologics standards.

Since few biological products can be standardized by chemical or physical means, an important activity of the Division is the establishment, evaluation, and distribution of "physical" biological standards for use by manufacturers and others engaged in biological standardization so that comparisons may be made between their products and standards of reference preparations provided by the Division. In this way, uniformity of potency can be assured and, in addition, a correlation maintained, where possible, with standards established in other countries, as well as with the international standards maintained under the auspices of the World Health Organization.

POLIOMYELITIS VACCINE TESTING

During the first eleven months of 1957, the testing of poliomyelitis vaccine continued to represent a major activity of the Division. More than 97 million cc's of vaccine were released during the year. One hundred and seventy-five lots of vaccine were tested for safety in monkeys and tissue culture, and potency determinations were performed on sixty-six lots. By November, the production and testing of poliomyelitis vaccine had reached the stage where the concept of mandatory "double testing" by the Division could be modified. Since

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then the procedure of testing individual lots of vaccine when there is indication for the need has been followed, as is done with other biological products. This will permit the Division to devote additional effort to other aspects of the poliomyelitis immunization problem--for example, the study of other poliovirus strains, the development of new tissue culture cell lines, and the study of attenuated live poliovirus vaccines.

CHICK POTENCY TEST

A new potency test for poliomyelitis vaccine, employing chicks instead of monkeys, has been developed by DBS scientists and the licensed manufacturers. The test is being used concurrently with the monkey potency test on a trial basis with a view to replacing the latter. This would be advantageous in terms of cost.

VACCINE TESTING BY COMPLEMENT-FIXATION METHODS

A supplementary method for assaying the potency of poliomyelitis vaccine is under study. Potency is at present measured in terms of the neutralizing-antibody response of monkeys inoculated with the vaccine. This procedure, while the only practical one available to date, has certain drawbacks from the standpoint of economy and facility. For instance, it is virtually impossible to use it for obtaining serial information on the changes in potency which might occur during the manufacture of a single lot of vaccine. In view of the relative speed and simplicity of the complement-fixation test, poliomyelitis vaccine and related preparations are presently being tested by this method under a variety of experimental conditions. Studies conducted in the Division's virus laboratories have shown that the test can be adapted to this purpose and used to discriminate between vaccines of high and low potency as measured in the standard monkey or chick assay.

GROWTH OF POLIOVIRUS IN CONTINUOUS CELL LINES

With the increasing difficulty in obtaining monkeys, it is essential that additional sources of cells for tissue culture work be obtained. One avenue that is being explored which may eventually obviate the necessity of using monkey kidney cells, at least for some purposes, is the use of serially cultivated self-perpetuating cell lines.

It has been found that certain lines of cells derived from the

rabbit, which become altered after a number of growth cycles, lose their natural resistance to poliovirus. However, at the time they become susceptible, the characteristic "rabbit" antigen disappears and an antigen common with cells derived from sources other than the rabbit manifests itself. The fact that this observation has now been repeated in other laboratories both in the U.S. and abroad suggests that it is a real phenomenon.

Much further work is necessary to characterize such altered cells before they may be utilized in the production of vaccines for human use.

OTHER VIRUS STUDIES

ADENOVIRUS VACCINE

After considerable testing, standards relating to the safety, purity, and potency of adenovirus vaccine--a biological product designed to prevent infections due to certain of the viruses which cause respiratory diseases--were drawn up by DBS scientists, given approval by the Surgeons General of the Army, Navy, and Public Health Service, and adopted as amendments to the official Regulations for Biological Products.

ASIAN STRAIN INFLUENZA VACCINE

The Asian strain influenza immunization program, which assumed national importance in 1957, involved both the control and the research potentials of the Division. During the first four months of production, beginning August 12, the Division tested and cleared for release 321 individual lots of vaccine. Through the use of ad hoc advisory groups and the continuous close cooperation with manufacturers' technical staffs as well as other experts in the field, numerous technical problems were successively overcome, and by mid-December over 61 million doses of Asian strain vaccine had been produced and cleared for release.

With confirmation that the influenza epidemic in the Far East this spring was due to a hitherto unknown strain of influenza virus, the Division procured samples of newly isolated strains for the six licensed manufacturers so that production of a vaccine to combat the new strain (Asian, type A) could be studied. As data became available suitable tests were developed, reference vaccines were established, data from laboratory, clinical, and field investigations were correlated, and specifications for the manufacture of a vaccine containing the

new strain were provided for the guidance of industry.

Since the characteristics of the strain suggested that a general epidemic could be expected to develop, there was an urgent and immediate need for an effective monovalent (Asian strain) influenza vaccine. The usual animal potency tests, which required considerable time for performance, were temporarily replaced by the chick cell agglutination (CCA) test which has been used in the past to measure the potency of influenza strains during production and in experimental situations. The CCA test is based on the capacity of influenza virus to cause clumping of chick cells in proportion to the amount of virus present. The test, however, while applicable to emergency situations is subject to many variables and will not replace the animal test in routine production.

Work is now under way to develop a simpler method for testing potency of influenza vaccine based on the same principle. In addition, a study is also being made of the behavior of influenza virus in tissue cultures with the aim of developing a clear-cut reproducible serum-virus neutralization test.

PROPAGATION OF MEASLES VIRUS

Since it is now possible to propagate measles virus in tissue culture, a study is under way to obtain data on the antigenic properties of living and inactivated measles virus from tissue culture sources. Virus is inactivated by various methods and its antigenicity determined. The response of man and animals to killed virus or measles infection is then measured. It has been found that antigenicity, as measured by the complement-fixation test, is retained when a variety of procedures are used to inactivate measles virus. These and other studies have been initiated so that the potency of a potential "measles vaccine" can be measured in a satisfactory manner should this vaccine become a practical biological product.

SMALLPOX VACCINE

Tissue culture methods are now being used experimentally in the standardization of smallpox vaccine. Serial cultures of a variety of tissue culture cells have been tested for their suitability. Although bovine, chicken embryo, and monkey kidney cells yield equal amounts of vaccinia virus following injection, monkey kidney cell culture appears to be the most suitable. Calf lymph vaccines which have been assayed by rabbit skin tests, or inoculation of fertile hens' eggs, can now be more precisely and conveniently evaluated by tissue culture plaque methods.

ISOLATION OF VIRUS-LIKE AGENT FROM MOUSE TUMORS BY TISSUE CULTURE METHODS

A study of mouse tumors, using tissue culture methods, has been carried out in DBS laboratories in collaboration with the National Cancer Institute. (See NCI Highlights.)

BLOOD PRODUCTS

LONG-TERM STORAGE OF BLOOD PRODUCTS

The stockpiling of red blood cells for transfusion purposes in the event of a national emergency, and the establishment of a central bank of rare blood cells, depend primarily on processing methods that permit long-term storage and shipment. The investigation of changes occurring in these products during storage which may affect their potency and safety is imperative in order to establish realistic dating periods and standards for storage of blood derivatives.

DBS scientists have found that red cells which have been stored in the frozen state can after thawing be kept at temperatures of 4° to 6° Centigrade for as long as ten days. These cells show a satisfactory in vivo survival, as demonstrated by radioisotope techniques.

A five-year study of the effects of storage on normal serum albumin in continuing. The material is stored under varying conditions and tested periodically for chemical and physical changes. The study has been in progress for 37 months and no evidence of major changes in the albumin have been demonstrated.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

BASIC AND GENERAL HEALTH PROBLEMS

1957

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the Division of Research Grants

The Division of Research Grants is a central unit responsible for the coordination and mechanics of the grant and fellowship programs of the National Institutes of Health. It is responsible for the receipt, processing, review, and payment of research grants, research training grants, research fellowships, and related extramural activities. Through the action of twenty-seven Study Sections and of several ad hoc committees, the Division is able to provide individual consideration for each grant application, and to make specific recommendations for action to nine National Advisory Councils. An equally important function of the Division is to stimulate, foster, and sustain a significant volume of research, principally in the basic biological and related sciences, not falling within the categorical responsibility of any of the Institutes.

During the past year, the biophysics research grant program was put into full scale operation; it serves to implement the program planning which took place the previous year. An international Institute is being planned for next summer by the Biophysics and Biophysical Chemistry Study Section for discussion of biophysical research. The world's authorities in physics, biology, and physical biology will meet to discuss present thinking, status, and trends in the field of physical biology. In this and other special programs, the Division is providing a continual stimulus and support for basic researchers in disciplinary, noncategorical investigations. This Study Section activity, conducted in close collaboration with the National Institute of Arthritis and Metabolic Diseases, will greatly assist this Institute in its plans for more intensive development of research in the field of physical biology.

Recognizing the need for trained personnel in critical research areas, the Division activated the General Research Training

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Grants Program. Its main purpose is to increase the number of trained scientists in such shortage areas as pathology, pharmacology, genetics, anesthesiology, epidemiology, biometry, biochemistry, biophysics and others from which come new basic knowledge vital to the conquest of disease. In addition to developments such as these, the Division continues to provide a focus for the multidisciplinary approaches that are involved in such fields as accident prevention, normal and abnormal mammalian development, and chronic toxicities due to ingestion of pesticides and food preservatives.

In those gap areas that are not of immediate concern to any of the Institutes, the Division continues to support fundamental research to give clinical investigators new tools for fresh, imaginative attacks against disease. Scientists are in total agreement that a reservoir of fundamental scientific knowledge must be maintained for sustained productivity in applied medical research.

Selected highlights of research progress during the past year are presented herein. They indicate not only accomplishments but new areas to be explored as a consequence of these findings.

ELECTRIC POTENTIALS OF THE HUMAN UTERUS

Grantees of the Division of Research Grants have succeeded in making a uterine electrical record of one complete labor, including delivery. This accomplishment of Dr. Sol D. Larks and his associates at the University of California is believed to be the first complete electrohysterogram of childbirth.

The electrical potentials of the human uterus in normal labor show a pattern of spikes and slow waves (or diphasic wave and slow wave pattern) synchronized with the uterine contraction and suggestive of the cardiac electrical complex in the low orders of animals with slow hearts. The duration of the complex shortens as labor progresses, and onset of rapid frequency signals early delivery. Mathematical analysis suggests the existence of a pacemaker, or possibly two pacemakers.

If this uterine electrical complex can be standardized, it will represent a major step in furthering our knowledge of uterine physiology and obstetrics. Studies such as this hold promise of providing the obstetrician with objective information to aid him in evaluation and diagnosis.

METABOLIC BREAK-THROUGH IN ENIGMA OF PSORIASIS

A new finding points the way to the solution of the enigma of psoriasis, a skin disease afflicting at least 4,000,000 persons in this country. It has already yielded a quantitative and objective test which promises to be a valuable diagnostic tool for the dermatologist.

Physical and chemical studies on the scaly skin that characterizes many types of psoriasis have revealed abnormal physical and chemical properties that are consistently measurable. The abnormal physical properties are reflected in the slow rates at which certain fluids pass through a column of pulverized scales and in the decreased water-binding capacity of these scales. The chemical anomalies encountered in all psoriatic scales studied so far are: (1) a low free amino nitrogen content, and (2) a high sulfhydryl content. Both anomalies are limited to the water-soluble fraction of the scales and are not found in the normal surrounding skin. The consistent combination of these chemical features offers a potential means of diagnosing the disease. Additional refinements are yet to be made before this test can become a routine diagnostic procedure.

These research findings by Drs. Peter Flesch and Elizabeth C. Jackson, grantees of the Division of Research Grants, are particularly valuable in that they shift the focus of attention from an unsubstantiated deficiency in the pancreatic secretion to a localized protein defect in the lesions themselves.

CHEMICAL STRUCTURE OF A PITUITARY HORMONE DETERMINED

In another study supported by the Division of Research Grants, Dr. Choh Hao Li and his colleagues at the University of California have determined the chemical structure of intermedin, a pituitary hormone, as isolated from pork and beef pituitary glands. When injected into frogs, the hormone causes the skin melanocytes to expand. This, in turn, changes the skin color of the frog from a light green to a darker hue.

Although this discovery has no immediate clinical import (the function of intermedin in man is unknown), it is an achievement of fundamental scientific importance. The investigators now know that intermedin and corticotrophin, another pituitary hormone, have a common structural "core". If all of the anterior pituitary hormones are structurally related, as is suspected, Dr. Li's work can well be said to constitute an important advance toward the synthesis of all known pituitary hormones.

ACCLIMITIZATION TO COLD ENVIRONMENT

Evidence presented by Dr. A. Kurt Weiss, a grantee of the Division of Research Grants at the University of Miami School of Medicine, suggests that the thyroid gland plays an important role in the successful adaptation of the animal organism to a cold environment. In his studies on the tissue responses of rats to low temperature, the investigator found that out of 10 tissues studied, only three definitely contribute to the over-all increase in the animals' oxygen consumption. These are liver, heart, and skeletal muscle.

Adaptation to cold apparently makes demands upon the pituitary and adrenal glands as well as the thyroid, since these glands hypertrophy in the cold-adapted animal. Removal of any of these glands decreases the animal's resistance to cold. Studies such as this are aimed to elucidate the many factors that enable the entire animal to acclimatize successfully to a cold environment.

ELECTRON MICROSCOPE REVEALS NEW PATTERNS IN CELL STRUCTURE

Electron microscope studies of animal cells have shed light on the mechanism by which the nucleus of a cell exercises control over the cytoplasm. Similar studies on plant cells suggest that the nuclear membrane of plant cells is fundamentally the same as that of animal cells, insofar as the structural pattern is concerned.

Previous research by grantees of the Division of Research Grants and other scientists has shown that the nuclear membrane is a double-layered fenestrated envelope with raised ring-like structures surrounding "pore-like" areas. Recent studies by Dr. Helen Gay, a grantee at the Carnegie Institute of Washington, have revealed chromosomal material in the blebs or outpocketings of these pore-like areas which suggest a mechanism for extrusion of chromosomal material from the nucleus into the cytoplasm. This extruded gene-carrying material is believed to regulate the biosynthetic (building-up) activities of the living cell.

Similar studies at the same Institute by Dr. K. B. De on the staminate and pollen cells of the plant, Tradescantia reflexa, suggest that the nuclear membrane of both plant and animal cells is fundamentally the same with respect to its structural organization. His finding narrows the chasm that separates animal and plant life at the cellular level.

NEAR-WASTE PRODUCT PROMOTED TO ESSENTIAL MEMBER ON ENZYME TEAM

Fundamental work on enzyme systems supported by the Division of Research Grants has shown that hypoxanthine, a nucleic acid derivative related to caffein and a precursor of uric acid in body metabolism, is a necessary component of the enzyme system by which the amino acid, cysteine, is oxidized to yield sulfate as one of its products.

Dr. Philip Handler, a grantee, has found that hypoxanthine is a hydrogen "transporter", taking hydrogen from the compound that is being oxidized and passing it on to diphosphopyridine nucleotide, another hydrogen transporter, or to the oxygen molecule itself. Hydrogen transporters are well known as necessary members of oxidative systems.

This is the first time a substance previously thought to be "on the way out" -- only two steps from the final waste product stage of uric acid itself -- has been identified as a necessary member of an enzyme team. The oxidation to sulfate of the sulfur in the sulfur-containing amino acids, cysteine and methionine, is the source of the sulfate found as a normal constituent in the blood and urine. This contribution to our knowledge of the basic mechanism by which the body incorporates elements into its tissues brings us closer to the core of many biological problems.

SENSITIVE TEST DETECTS DIABETIC DANGER SIGNALS

A new quantitative test, more sensitive than the one now commonly used, has been developed to measure the quantity of acetone bodies in blood and urine. The test is an offshoot of fundamental studies in the quantitative microdetermination of certain organic compounds. Basic studies by Russian scientists in 1937 enabled Dr. Victor E. Levine and his associates at Creighton University School of Medicine to perfect this quantitative method.

The new test, which utilizes vanillin in an alkaline medium, offers many advantages over the widely used quantitative test of Behre and Benedict in which salicylaldehyde, also in alkaline medium, is used as the reagent. A major advantage of the grantees' method is found in the greater stability of vanillin solution over salicylaldehyde which deteriorates and discolors very rapidly.

Furthermore, in the Behre and Benedict test, the addition of the acetone brings about an increase in a color already present, whereas in the grantees' method the solution is colorless until

the acetone is added. This difference between the two methods permits greater accuracy in the measurement of the acetone present.

Any increase in the acetone in the urine of a diabetic is a danger signal of possibly impending coma. In the diabetic child, development of ketosis and accompanying coma is the most serious hazard to his life. For this reason, clinicians urge diabetics to examine their urine frequently for acetonuria.

FACTORS INFLUENCING SUCCESSFUL SKIN GRAFTS

Studies on laboratory animals have shown that the immune mechanism of the host can be made more tolerant of skin grafts when living cells from the future donor are injected intravenously into the embryonic future host. At the University of California Medical Center, Dr. Jack Cannon, a grantee of the Division of Research Grants, is continuing these studies with emphasis on the many related factors that influence a successful "take" in skin grafting.

Dr. Cannon and his colleagues have perfected a technique which permits cross-transfusion of blood between embryo chicks with negligible mortality. Interchange of blood prior to hatching enables subsequent skin grafts to survive for longer periods of time than grafts in chicks receiving cross-transfusions after hatching. With this method approximately 40 percent of the 60 two week old chicks that were cross-transfused prenatally showed graft survival six months after grafting. In 89 untreated chicks, also two weeks old when grafting was performed, no grafts survived after five weeks. Upon cross-transfusion of blood shortly after hatching, skin grafts "took" initially in 40 percent of the chicks, but dropped to 2.5 percent by six months. It was further demonstrated in this study that the percentage of "take" increased when larger quantities of blood were transfused.

Studies of this kind are providing important data that will, it may be hoped, enable surgeons to achieve uniformly successful skin grafts and gain further insight into the many factors that must be considered for successful transplantation of human organs.

NEW SURGICAL TECHNIQUE PROLONGS RELIEF IN ESOPHAGEAL DISORDERS

Lasting relief in certain spastic disorders of the esophagus and stomach is now possible as a result of a new surgical technique devised by a gastroenterologist at the University of Minnesota

Medical School. By modifying and improving an operation known as the "extramucosal myotomy of Heller", Dr. Owen H. Wangensteen has achieved prolonged relief in achalasia of the esophagus and cardiospasm.

Subsequent to the original Heller procedure, patients are able to ingest food -- a difficult feat in cardiospasm; but, emptying of the esophagus is delayed and may be incomplete. Dr. Wangensteen's operation, the result of five years' study supported in part by the Division of Research Grants, achieves complete emptying of the esophagus.

In its essentials, the operative technique involves distention of the circular muscle fibers of the esophageal wall by means of a balloon-tipped catheter. Complete disruption of these fibers is ensured by cutting all resistant muscular bands. The operation is recommended in all cases where non-surgical dilatation of the spastic, hypertrophied esophagus is unsuccessful.

BACTERIAL ANTI-GROWTH FACTOR INHIBITS FUNGUS GROWTH

Lipoic acid, a non-metabolite known to inhibit metabolism in certain bacteria, was shown to retard growth of the watermold, Allomyces macrogynus. The finding is potentially useful in the control of fungi.

The specific inhibitory effect is on the "lag phase of growth", the early period of growth following inoculation of the mold into a culture medium. Data presented by Dr. Leonard Machlis of the University of California indicate that lipoic acid displaces glutamic acid, an amino acid (a building block of protein) essential in the metabolism of some fungi, bacteria, and insects.

The inability of certain bacteria to distinguish between essential nutrients and their corresponding antimetabolites is of growing importance in clinical medicine. For example, p-aminobenzoic acid (PAB) is required in the metabolism of certain streptococci. Sulfonamides, which are structurally related to PAB, successfully compete with it in the metabolism of these bacteria and thus inhibit their growth.

CONGENITAL ANOMALIES INDUCED BY IMMUNOLOGICAL MECHANISMS

Nervous system abnormalities were induced in embryonic mice whose mothers were treated with mouse brain emulsions prior to pregnancy. These findings

demonstrate that immunological mechanisms (maternal antibodies) act directly on the processes involved in the development of embryonic organs.

Dr. Salome Gluecksohn-Waelsch of the Albert Einstein College of Medicine injected normal female mice with emulsions of adult mouse brain and then bred them through brother-sister matings. Abnormalities of the nervous system were found in a high percentage of embryos. These consisted of suppression of nervous tissue differentiation in the region of the brain and anterior spinal cord, microcephaly and abnormalities of closure of neural folds. As controls, siblings of brain-injected animals were injected with heart emulsion. None of the offspring showed any abnormality of the nervous system.

These findings support the hypothesis that immunological mechanisms (antigen-antibody reactions) may interfere with the normal development and differentiation of embryonic organs. This is one of several studies supported by the Division of Research Grants which are directed toward the solution of the problem of congenital anomalies. These account for 70,000 infants yearly so crippled by prenatal damage that they do not survive the first four weeks of life. Another 150,000 survive their prenatal or birth damage but remain handicapped throughout their lives.

CHEMICAL STRUCTURE OF ALFALFA CONSTITUENT DETERMINED

Scientists at Wayne University have isolated medicagenic acid from alfalfa and determined its chemical structure. Medicagenic acid is related to the saponins, foaming soap-like plant substances, some of which are toxic when eaten by chicks and ruminants. Saponins are characterized by their ability to rupture red blood cells even in high dilutions.

This fundamental study conducted by Dr. Carl Djerassi and his associates may be of immediate practical significance because the mixture of saponins which occurs in alfalfa is known to produce deleterious effects in chicks and ruminants. It may aid also in the finding of less complicated, physiological active steroid molecules related to the saponins.

SKIN BACTERIA YIELD ANTIBIOTIC EFFECTIVE AGAINST GANGRENOUS INFECTION

An antibiotic prepared from a commonly found skin organism, Staphylococcus albus, has been shown to be highly effective in protecting animals against lethal inoculations of Clostridium septicum, the organism that produces gas gangrene.

Studies by Dr. Seymour P. Halpert and associates at Presbyterian Hospital, New York City, have demonstrated the remarkable efficacy of this antibiotic. Even, when administered as late as six hours after injection of the infecting organisms, the antibiotic prevents a fulminating infection that without it kills mice within 18 to 24 hours. Although the data presented are not extensive enough for precise comparisons, they suggest that the staphylococcal antibiotic has about the same prophylactic activity as penicillin or oxytetracycline, the latter a broad spectrum antibiotic.

On the basis of these observations, it would appear that bacteria "normally" associated with human beings are potential sources of clinically useful antibiotics. It is pertinent to note that these findings stem from fundamental ecological investigations, supported by the Division of Research Grants, of antibiotic-producing organisms found in human beings.

FUNDAMENTAL STUDIES ON AIRBORNE BACTERIA YIELD "PRACTICAL" DIVIDENDS

Investigations into the fundamental nature and behavior of airborne bacteria have demonstrated the presence of a protective capsule of non-living material from the culture medium when these bacteria are atomized from a culture. That such a protective capsule can prolong the survival of these comparatively "naked" bacteria after the use of aerial disinfectants has practical implications.

Other studies have demonstrated the usefulness of these bacteria, Serratia marcescens, as "tracers" in aerosol studies. Aerosols serve a variety of purposes--from spray drying to insect killing. The introduction of bacteria into aerosols has enabled the investigators to evaluate the performance of atomizers and the particle size of dispersed solutions or suspensions and to determine the homogeneity of dispersion of aerosol clouds.

Other uses of this viable research tool now include a method for testing air filters and other forms of arresters; detection

of trace vapors and gaseous air pollutants; and tracking the meterological dispersion of pollutants from smoke stacks.

WOOD CONSTITUENT FOUND TO DECREASE GASTRIC SECRETION

Preliminary studies with calcium lignin sulfonates from pulp mill sulphite waste liquor demonstrate that these compounds reduce gastric secretion and counteract experimental measures taken to produce gastric ulcers.

Screening tests by grantees of the Division of Research Grants to determine the gastric anti-secretory activity of various compounds reveal that calcium lignin sulfonates afford considerable protection against laboratory-induced gastric ulcers in rats.

In a large screening operation, Dr. T. Lloyd Fletcher and his colleagues at the University of Washington School of Medicine, studied the effects of lignin sulfonate derivatives on gastric secretion in pylorus-ligated rats. Previous studies have shown that ligation of the pylorus produces gastric ulcers within 24 hours. Such rats treated with calcium lignin sulfonates were free from ulcers and had significantly less gastric secretion than the control rats. Although the tests are exploratory in nature, they are of fundamental importance, adding to our knowledge of gastric physiology and pointing the way to better treatment of peptic ulcer.

TREATMENT OF LEAD POISONING

The use of lead-based paint is a constant health hazard, especially in older houses where there is crumbling and peeling of the window and door finishes. Young children are known to eat or gnaw such surfaces and thereby may become victims of lead poisoning.

As a result of biochemical and pathological studies by Drs. Julian Chisolm, Jr., and Harold E. Harrison of Johns Hopkins University School of Medicine, a more effective plan of treatment for lead poisoning has been suggested. Essentially, it consists of therapy with edathamil calcium disodium in conjunction with specific supportive management during the first 48 to 72 hours. Edathamil calcium disodium, known to chemists as a chelating agent, increases the urinary output of the accumulated lead.

Some of the symptoms of lead poisoning are mental with behavior

patterns indicating central nervous system damage. According to the investigators whose work has been supported by the Division of Research Grants, permanent mental damage is less likely to develop if patients are given repeated courses of treatment with the chelating agent and are relocated to prevent re-exposure to lead.

BACTERIAL ACTIVITY IN INDUSTRIAL OILS

Bacteria are found in most soluble oil emulsions and synthetic cutting fluids used in industry. They are believed to be the cause of foul odors and staining of metals, and may even be the cause of dermatitis, since some of these organisms are known to be potentially pathogenic. Studies at the University of Nebraska have shown inadequacy in the methods of disinfection of lubricating and cooling oils currently used by the manufacturers.

Bacteria isolated from these solutions, grown in pure culture and injected into mice, were found to be 50 to 100 percent lethal, depending on the specific bacteria involved. Current studies indicate that many factors need to be understood before bacterial activity in these oils can be brought under control. Drs. Hilliard Pivnik and C. K. Fotopoulos, grantees of the Division of Research Grants, are continuing their investigations in the following fundamental areas: (1) Study of the taxonomy and physiology of bacteria isolated from soluble oil emulsions, (2) Growth of pathogenic bacteria which cause typhoid fever and related enteric diseases, (3) Disinfection of soluble oil emulsions, (4) Oxidation of soluble oil emulsions and components of soluble oils, and (5) Reduction of inorganic sulfur compounds in soluble oil emulsions.

ANNELID EGGS HIGHLY USEFUL IN RADIATION STUDIES

Recent concern over diagnostic x-ray hazards and radiation fall-out resulting from nuclear detonations reemphasizes the importance of fundamental studies aimed to shed light on the effects of ionizing radiation on living systems.

One such study by Drs. Donald P. Costello and Catherine Henley of the University of North Carolina has demonstrated that the eggs of the marine annelid, Chaetoperus pergamentaceus, are highly suitable for study of the effects of irradiation on the early stages of development of this organism. Relatively

low doses (255 r. to 765 r.) caused retardation of cell cleavage, ciliary defects, cytoplasmic blebs and feeble movements. Numerous abnormalities were also observed within the nuclear bodies that determine the hereditary pattern of the annelid. Somewhat higher doses (1020 r. and above) caused death in the larval stage.

HIGHLIGHTS OF PROGRESS

IN SUPPORT OF RESEARCH

AT NIH

1957

Items of Interest on Program Developments and Research Studies

Conducted by the Division of Research Services

In the Division of Research Services, 800 persons with almost 200 varying levels of occupational skills provide the scientific, technical, and engineering support required by the concentrated research effort at the National Institutes of Health.

This support involves the manifold services and talents of instrument makers, engineers, biometricians, veterinarians, scientists, librarians, translators, editors and writers, medical photographers, scientific illustrators, and many others.

Frequently the support of research demands basic research itself. In this respect, DRS scientists and technologists work side by side with Institute investigators in order to design and develop the special instruments, apparatus, and materials needed in the course of individual research projects. Generally, such collaboration results in the joint preparation of a paper for publication.

Additionally, in an effort to steadily raise the quality level of DRS services, the Division anticipates needs and initiates studies over and above those specifically requested. Such independent Division projects during 1957 included a study of the NIH distilled water system in order to improve and permanently control water quality; studies of animal housing and feeding procedures in order to produce better quality experimental animals; and a genetic study to develop a hardier strain of rat for use in research. (The latter study is reported herein.)

Following are some of the Division's specific accomplishments during the past year in technical, research, and developmental support at NIH.

COLOR MOTION PICTURES OF CIRCULATORY SYSTEM IN BRAINS OF LIVING ANIMALS

Photography Section of Scientific Reports Branch, working with NINDB investigators, produced for the first time clearly differentiated color motion pictures of the traumatized and untraumatized circulatory systems of the brains of living experimental animals, and still color photographs of fluorescent dyes permeating the brain tissues of sacrificed animals.

Motion pictures of the brain's circulatory system in the normal and pathological state were obtained through the use of very high speed film in a standard motion picture camera, and by employing a color-corrected and highly concentrated light source. The light source was an extremely brilliant daylight "spot". Still color photographs of the same areas fluoresced with special dyes were obtained through the use of ultraviolet light.

NINDB's Section on Clinical Pathology anticipates that successful production of motion pictures of normal and pathological brain tissues will prove of future value in the operating room as a means of documentation and diagnosis. The investigators are employing the fluorescence technique in studies of the blood-brain barrier.

PLASTIC HEART VALVES

Technologists of the Medical Arts Section, Scientific Reports Branch, and a cardiac surgeon of the National Heart Institute collaborated to produce workable plastic heart valves made of silicone rubber.

Technical difficulties in fabricating the valves were two: selecting and blending non-toxic, malleable plastic that would maintain rigid or flexible surfaces as desired; and designing and producing the dies in which the valves were ultimately molded.

In NHI, a plastic aortic valve, surgically inserted in a dog's heart, functioned successfully for 10 hours. There was no evidence of blood clotting, and no fibrin was deposited on the valve. Other test animals receiving plastic mitral valves survived up to five days.

FIRST DUAL RADIOISOTOPE SCANNER AT NIH

NIH's first dual radioisotope scanner has been fabricated by Instrument Section, Laboratory Aids Branch. There are few scanners of this type in use in the United States today. NINDB investigators will employ the scanner in detection of tumors or cancerous tissues in the brain. Key to the instrument's fabrication was the successful production of an intricately designed solid (99%) gold collimator to eliminate stray radiation. Machining the collimator required special cutting tools and fixtures.

PLASTIC HEART PUMP

A new plastic heart pump which simulates as closely as possible the action of the heart has been produced through the collaboration of Medical Arts Section, Scientific Reports Branch, and a cardiac surgeon of the National Heart Institute.

The heart pump features two separate systems: one pumps blood through the lungs; the other, through the body's peripheral vessels.

Tested on a dog, the pump successfully maintained the animal's normal pulse rate and blood pressure for two and one-half hours. The blood

was satisfactorily circulated and oxygenated, and there was no observable damage to the blood cells.

An advantage of the new pump over those now in use is that it does not require priming with whole blood.

GERM-FREE LABORATORY ANIMALS FOR RESEARCH

Sanitary Engineering Branch, working with investigators of NIAID, NIAMD, and NIDR made significant progress in developing, maintaining, and improving the germ-free animal facility established at NIH in November 1956.

Initially put into operation with eight chambers, the facility now offers 16.

During 1957, investigators of the three Institutes employed the chambers in 39 complete cycles. Additionally, scientists of the Section on Germ-Free Animals Studies, NIAID, delivered 276 guinea pigs in the units. One chamber was set aside for breeding rats, and a number of scientists and technicians were trained in chamber techniques.

SEB personnel developed the facility to accommodate the original equipment, designed at Notre Dame. Since that time, SEB has developed improved techniques, materials, and equipment that have materially increased the efficiency and reliability of the germ-free operations.

A major SEB objective in this program is to facilitate the use of germ-free animals in NIH research by developing a system that will supply animals directly to the laboratories in portable, economical, adaptable, and easily operated chambers.

TRANSLATION OF RUSSIAN SCIENTIFIC INFORMATION PROGRESSES AT NIH

The broad program for translating and disseminating Russian scientific information in the medical and biological sciences achieved most of the program's primary objectives during 1957.

Established in 1956, the program is a function of the Library Section, Scientific Reports Branch, and is coordinated with similar programs of the National Science Foundation and the Atomic Energy Commission. It is administered through grants and contracts.

Accomplishments of the NIH program during the past year include the translation and distribution of 600 copies of a "Directory of Soviet Research Institutes in Biology and Medicine"; 500 copies of Koenig's "Medical Research in the Soviet Union: a Selected and Annotated List of References"; and 1500 copies of a "Guide to Scientific Translations Services." (The latter is PHS Publication No. 514. A new edition is in preparation at the Government Printing Office.)

Further accomplishments are the translation and distribution of 27 issues of eight journals (containing 583 Russian scientific papers) to 300 medical libraries and 80 Government installations; publication and distribution of six issues of "Abstracts of Soviet Medicine," containing approximately 3000 abstracts; and translation of 56 individual Soviet papers for screening.

A study of the organization and functions of the Academy of Medical Sciences, USSR, has been edited for publication, and a Russian-English medical dictionary brought to the final editing stage.

Jointly with the National Science Foundation, NIH provided grant support for the national translations pool at the John Crerar Library, Chicago, and maintained close liaison with other Federally supported translation activities.

CLINICAL CAMERA ACCURATELY RECORDS TISSUE COLOR CHANGES

Technologists of the Photography Section, SRB, and Instrument Section, LAB, have collaborated with clinical investigators of the CC Dental Department and NIDR to produce a "clinical camera" to accurately measure and permanently record color changes in living tissue. The camera is now under construction. At present, there is no equipment for measuring and recording such information.

The camera is a semi-automatic 70-millimeter, equipped with a filter wheel containing seven red filters that range in density from light to dark. When started, the camera automatically indexes itself eight times: the first exposure is an unfiltered photograph of the tissue under study; the remaining seven exposures range the filter wheel. Each exposure is made through a different filter.

Simultaneously, through a separate optical system, a "gray scale" is printed across the upper margin of each of the seven negatives. The "gray scale" provides insurance for the future reading of a fading negative. Since the "gray scale" and the photograph of the tissue are placed on the negative through two separate optical systems, a densitometer (by comparing the two separate readings of the "gray scale" versus the photograph) can give the negative's original reading.

The purpose of the filter wheel is to find the color density of the tissue being photographed. When the filter density matches the color density of the tissue, the tissue "blends" with the filter color and cannot be separately distinguished on the negative.

By means of the densitometer, dental investigators believe it will be possible to accurately assign a color index of the original tissue and of the tissue's subsequent changes. The camera may also have application in eye diseases, dermatology, and pathology.

HARDIER STRAIN OF RATS FOR RESEARCH

Geneticists of Animal Production Section, Laboratory Aids Branch, have been researching the breeding of a new, hardier strain of rat for use in research. Indications are that progress is being made toward developing a rat strain that is more resistant to respiratory infection and to lung lesions. Both conditions are frequently found in other laboratory rats, and severely limit the use of the rodents as experimental animals.

The new rats, bred by a series of backcross matings of NIH Black and Sprague-Dawley strains, have been tested in NIH laboratories with satisfactory results.

Successful production of a rat strain which manifests all the characteristics most desirable for research use will result in lower maintenance and production costs for rat colonies, and will provide the scientist with a better quality research tool from which more dependable data may be obtained.

TITANIUM PROSTHESIS PROVES SUCCESSFUL

An investigator in the Animal Hospital Section, Laboratory Aids Branch, has successfully demonstrated on dogs that a titanium prosthesis can be used as a replacement for injured bone shaft, and to lengthen bones that have been shortened by injury. The prosthesis is contained entirely within the medullary canal.

This is the first instance in which titanium has been used in a prosthetic device for lengthening the long bones.